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A STUDY TO ESTABLISH RELATIONSHIP OF INSULIN RESISTANCE AND ANTI THYROID PEROXIDASE ANTIBODIES IN HYPOTHYROID PATIENTS OF SUBHIMALAYAN REGION OF WEST BENGAL

Biochemistry

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ABSTRACT

The prevalence of thyroid disease in patients with diabetes is significantly higher indicating a possible interplay between thyroid status and insulin sensitivity. Significantly raised prevalence of elevated fasting insulin as well as Anti TPO Ab is detected in a subgroup of patients with Hashimoto's thyroiditis and might support the role of autoimmunity in the development of insulin resistance. The proposed location of this study is the Darjeeling district of West Bengal. The endeavor of this study is to substantiate the relationship between Anti-TPO antibody levels and Insulin resistance if any with hypothyroidism. An institution-based observational cross-sectional study consisting of 122 hypothyroid males and female (20-55 years) subjects. Thyroid function test, fasting insulin, fasting glucose, and anti-thyroid peroxidase antibody (anti-TPO) estimation were done. HOMA-IR (homeostatic model assessment insulin resistance) was calculated. 39% of subjects were anti-TPO-Ab positive (cut off >=35IU/ml) whereas 47% were with positive insulin resistance. Anti-TPO Ab positive subjects had higher mean values of insulin resistance (HOMA-IR) in comparison to anti-TPO Ab negative subjects (HOMA-IR – 3.72 ± 0.97vs 2.15 ± 0.49)] with p <0.001(Unpaired Student's t-test). Bivariate analysis showed that Insulin resistance was positively correlated with Anti-TPO Ab status in anti-TPO Ab positive group with p-value 0.004, <0.001 and <0.001 respectively. Correlation between anti-TPO-Ab status with HOMA-IR is highly significant in the anti-TPO positive group which records a strong association between thyroid autoimmunity with insulin resistance for developing diabetes mellitus.]

KEYWORDS

INTRODUCTION

Thyroid diseases are one of the most common endocrine disorders worldwide. India too is no exception. According to projections from various studies on thyroid disease, it has been estimated that about 42 million people suffer from thyroid disease. [1] It is now widely accepted that the thyroid diseases traditionally known as lymphocytic thyroiditis and Hashimoto's thyroiditis represent different phases of manifestations of an organ-specific immune-mediated inflammatory disorder designated as autoimmune thyroiditis and characterized functionally by the production of autoantibodies that alter thyroid function. [2-5]

The mechanisms leading to autoimmune thyroiditis are both humoral and cellular. [6-9] Circulating autoantibodies exist against thyroglobulin and other follicular cell antigens. [5] However, it has been suggested that the initial factor resulting in autoimmune thyroiditis is an organ-specific defect in suppressor T- lymphocytes. The available evidence suggests that autoimmune thyroiditis is under the influence of multiple genes and multifactorial, and thus of variable penetrance. [10]

Lymphocytic thyroiditis is the most prevalent cause of subclinical or overt hypothyroidism in areas with sufficient iodine intake. [11] The disease is characterized by the presence of anti-thyroid peroxidase (anti-TPO) antibodies. Microsomal antibodies are present in 95% of cases. The annual risk of developing clinical hypothyroidism is 4% when subclinical hypothyroidism is associated with positive thyroid peroxidase (TPO) antibodies. [12]

Thyroid hormones play an important role in regulating energy balance, metabolism of glucose, and lipids. Sub-clinical hypothyroidism and overt hypothyroidism are associated with insulin resistance, dyslipidemia, hypercoagulability, and low-grade inflammation. [13]

Insulin resistance is the cardinal feature of type 2 diabetes mellitus. The main pathophysiological basis underlying glucose intolerance, dyslipidemia, central obesity, and hypertension has been attributed to insulin resistance. [14]

Though the processes by which insulin resistance develops are incompletely understood, the increased plasma concentration of inflammatory mediators (such as tumour necrosis factor-α and interleukin-6) is associated with insulin resistance. [15]

The prevalence of thyroid disease in patients with diabetes is significantly higher than that in the general population. [14] This indicates a possible interplay between thyroid status and insulin sensitivity or resistance. [14] Thyroid autoimmunity can cause several forms of thyroiditis and abnormal thyroid functions, ranging from hypothyroidism to hyperthyroidism. [16] Furthermore, anti-thyroid peroxidase antibody (anti-TPO antibody) is a member of thyroid autoantibodies, which are important in inducing and also diagnosing autoimmune thyroid diseases. [16] A significantly more prevalence of elevated fasting insulin level is detected in a subgroup of patients with Hashimoto's thyroiditis and highly elevated levels of Anti-TPO antibodies might support the role of autoimmunity in the development of insulin resistance. [17]

The proposed location of this study is situated at Darjeeling district of West Bengal which is a part of the sub-Himalayan terai region with a mixed population. A high prevalence of thyroid dysfunction is present in this area which is unique in this manner. [1] The endeavor of this study is to substantiate the relationship between Anti-TPO antibody levels, lipid profile, and Insulin resistance if any in-patient with hypothyroidism. These may help to prevent the premature development of metabolic syndrome as well as diabetes in advance.

AIMS AND OBJECTIVES

- To measure serum Anti TPO antibody and insulin resistance by HOMA-IR in hypothyroid subjects.
- To detect the degree of the association if any between titers of Anti-TPO Ab and insulin resistance in the above-mentioned group.

MATERIALS AND METHODS

In this Institution-based observational cross-sectional study, 122 individuals, both male, and female, age between 20 -55 years, fulfilled the diagnostic criteria of hypothyroidism attending the outpatient department of North Bengal Medical College and Hospital and referred to both the central clinical laboratory and laboratory of

Department of Biochemistry for routine tests and thyroid profile between April 2016 to March 2017 were taken as study subjects. Sampling was done by simple random sampling method. Detailed history taking and clinical examination were done. Thyroid function tests including fT4, TSH, Anti-TPO Ab, and Insulin were done by the ELISA method. FPG is measured by glucose oxidase peroxidase methods.

Insulin resistance was calculated by HOMA IR criteria with the help of the following formula.

$$HOMA\ IR = \frac{Fasting\ Plasma\ Glucose(mg/dl)\ x\ Fasting\ serum\ Insulin\ (IU/L)}{405}$$

For study purposes normal reference range of thyroid hormones will be considered as follows: fT4 0.8 – 2 ng/dl. TSH 0.39-6.16 µIU/L depending on the ELISA kit used.

The individual will be considered as in hypothyroid state if TSH>6.16 µIU/L.

The normal range for fasting insulin is taken to be 5–19 µU/mL. [Kit manual]

The cut-off level for Anti-TPO Ab is set at 35 IU/ml.[18]
Insulin resistance cut off value is set as HOMA-IR 2.5[19]
No control is required for this study.

RESULTS AND DISCUSSION

Collected data were processed, enlisted, tabulated, and analyzed using statistical software IBM SPSS statistics version 22 and Microsoft Excel 2016.

From our study, it is observed that the maximum number of subjects (31%) were in the 35 to 41-year age group. Among the total number of 122 subjects, 24 (20%) were male and 98 (80%) were female. 48 (39%) were Anti-TPO Ab positive and 98 (61%) were Anti-TPO Ab negative. The ATPO-Ab positive group contains 7% male and 32% female whereas Anti-TPO Ab negative group contains 12.3% male and 48.7% female **(Figure 1)**. Amongst the total subjects, 48 (47%) were Insulin Resistance positive and 74 (53%) were Insulin Resistance negative. The insulin resistance positive group contains 7.4% male and 41.8% female whereas Insulin resistance negative group contains 12.3% male and 38.5% female **(Figure 2)**.

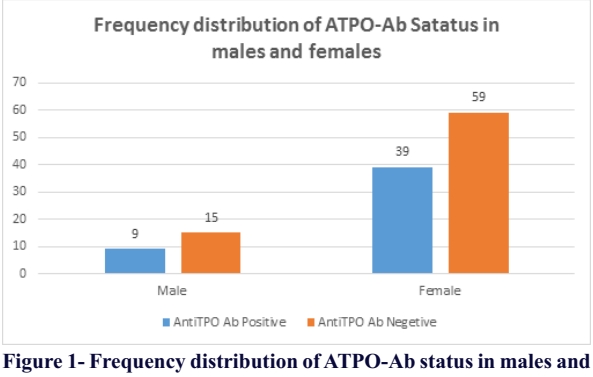

Figure 1- Frequency distribution of ATPO-Ab status in males and females

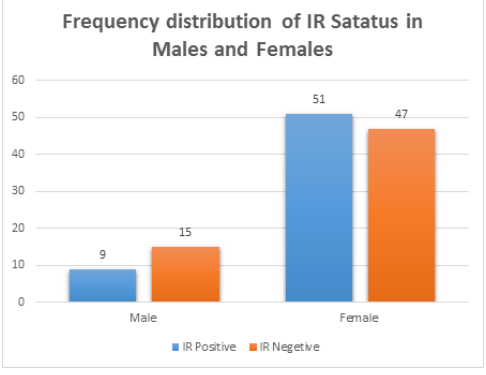

Figure 2 - Frequency distribution of Sex by IR Status

Many studies (conducted by Fairweather D et al, The Whickham Survey, A.G. Unnikrishnan, et al., Aryal M et al.) sketched the effect of age and gender on the prevalence of autoimmune diseases. For most autoimmune diseases there were clear sex differences in prevalence whereby females were generally more frequently affected than males.[20-24]

A.G. Unnikrishnan et al. found that compared with young adults (aged 18-35 years), older adults had greater chances of being diagnosed with hypothyroidism. The prevalence of hypothyroidism was found to be highest in the age-group of 46 to 54 years (13.11%) and the lowest in that of 18 to 35 years (7.53%), in their study conducted in eight major cities of India.[23]

Aryal M et al. in their study showed that females and advanced aged people were more vulnerable to thyroid dysfunction in the population.[24] This is in accordance with the present study.

Fairweather D et al. proposed that women were known to respond to infection, vaccination, and trauma with increased antibody production and a more T helper (Th)2-predominant immune response, whereas a Th1 response and inflammation are usually more severe in men.[20]

Table 1 Comparison of the mean values of TSH, fT4, FBS, Fasting Insulin, IR between anti-TPO Ab Positive and anti-TPO Negative groups (unpaired Student's t-test)

Parameters	Anti TPO Positive (Mean±SD)	Anti TPO Negative (Mean±SD)	t value	p-value
TSH (µIU/ml)	19.45 ± 4.20	14.60±4.13	6.286	< 0.001**
fT4 (ng/dl)	0.80 ± 0.20	0.98 ± 0.24	-4.115	< 0.001**
FBS (mg/dl)	95.00 ± 7.49	92.94 ± 8.13	1.412	0.161
Fasting Insulin (µIU/ml)	15.82 ± 3.84	9.44 ± 2.33	11.422	< 0.001**
IR(HOMA-IR)	3.72 ± 0.97	2.15 ± 0.49	11.798	< 0.001**

Table 2- Showing correlation between Anti TPO Ab & following parameters (TSH, fT4, FBS, Insulin, IR) in Anti TPO Ab + group (anti TPO Ab> 35 IU/ml) (Pearson correlation)

Anti TPO Ab Positive		TSH	fT4	FBS	Insulin	IR
	Correlation coefficient(R)	**0.504**	**-0.394**	**0.411**	**0.697**	**0.764**
	Significance (p) (2- tailed)	<0.001**	0.006*	0.004*	<0.001**	<0.001**

In the present study anti-TPO, Ab positive subjects had higher mean values of TSH and the lower mean value of fT4 in comparison to anti-TPO Ab negative subjects [(TSH-19.45 ± 4.20 vs 14.60 ± 4.13) µIU/ml, (fT4 – 0.80 ± 0.20 vs 0.98 ± 0.24) ng/ dl]. **(Table-1)**

Unpaired Student's t-test showed that there was a significant difference between the mean values of serum TSH and fT4 respectively between the anti-TPO positive and anti-TPO negative group with the $p < 0.001$. **(Table-1)**

From the bivariate analysis, it was observed that TSH and fT4 both were correlated with Anti-TPO Ab status In Anti-TPO positive group. TSH was positively correlated whereas fT4 was inversely correlated with the p-value <0.001 and <0.006. **(Table-2)**

The present study also showed that anti-TPO Ab positive subjects had higher mean values of FBS, fasting Insulin and Insulin Resistance (HOMA-IR) in comparison to anti-TPO Ab negative subjects [(FBS-95.00 ± 7.49 vs 92.94 ± 8.13) mg/dl, (Insulin - 15.82 ± 3.84 vs 9.44 ± 2.33) µIU/ml, (HOMA-IR – 3.72 ± 0.97vs 2.15 ± 0.49)]. **(Table-1)**

Unpaired Student's t-test also showed that there was a significant difference between the mean values of fasting Insulin, insulin resistance with p<0.001. But there is no significant difference between the mean values of fasting blood sugar among the two groups as the p-value is calculated to be 0.16 and significance is considered at the p-value < 0.05. **(Table-1)**

On the other hand, from the bivariate analysis it was observed that FBS, Insulin, and Insulin resistance were positively correlated with Anti-TPO Ab status in Anti-TPO positive group with the p-value 0.004, <0.001, and <0.001 respectively. **(Table-2)**

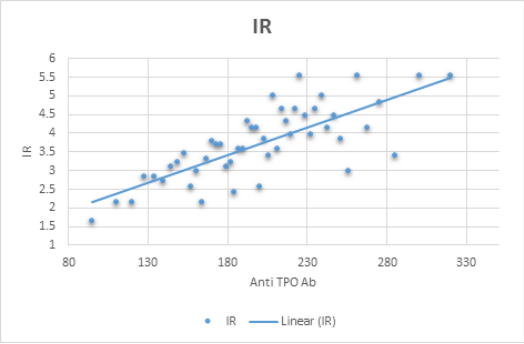

Figure 3 Linear Regression line for Serum Insulin Resistance with Serum Anti-TPO Ab among patients with positive anti-TPO Ab status

Table 3 Showing the correlation between Anti TPO Ab & following parameters (FBS, Insulin, IR, BMI) in Anti TPO Ab negative group (anti-TPO Ab < 35 IU/ml) (Pearson correlation)

Anti TPO Ab negative		FBS	Insulin	IR	BMI
	Correlation coefficient (r)	0.107	-0.107	-0.058	0.057
	Significance(p) (2- tailed)	0.366	0.362	0.622	0.630

The findings further extend to the fact that anti-TPO Ab negative subjects (61%) did not show any correlation between anti-TPO Ab level and serum insulin or HOMA-IR level as evidenced by bivariate correlation. This finding further strengthens the observation of the present study. **(Table-3)**

Hypothyroidism could be considered as T helper1 disease in which pro-inflammatory cytokines such as TNF- α and IL-6 play a crucial role. There is a connection between the level of Anti-TPO Abs and pro-inflammatory cytokines like TNF-α and IL-6. The antibodies produced against the antigens specific to the thyroid like TPO, result in an immunity complex, which activates the complement pathway and ultimately the T cells, thus leading to an increase in the production of pro-inflammatory cytokines Therefore, Anti-TPO Abs, which plays a predictive role in the progress of hypothyroidism can have either a direct cytotoxic effect for thyroid cells through the IgG1 class or an indirect destructive effect on thyrocytes through activating TH1 cells and increasing inflammatory responses via the release of inflammatory cytokines. Besides, the secretory function of monocytes and lymphocytes is associated with the Anti-TPO Abs titer which can be accounted for by the fact that the interaction between the monocytes and T cells activates B lymphocytes and produces Anti-TPO Abs. Monocytes, lymphocytes, and cytokines produced by these cells play an essential role in triggering autoimmunity disorders. The monocytes, macrophages, lymphocytes coupled with the cytokines secreted by these cells – in particular, TNF-α and IL-6 – contribute to insulin resistance and atherosclerotic plaques.

In recent years, tremendous interest has been raised in the effect of thyroid function on insulin levels. It is established that clinical hypothyroidism is considered an insulin-resistant state. [25,26] Following their studies on patients with hypothyroidism, Handisurya [27] and Stanicka [28] have concluded that hypothyroidism makes glucose inaccessible to insulin. [15]. However; an exact pathogenetic mechanism involved in insulin resistance in hypothyroidism is still unknown. In the current study, we tried to detect any association of autoimmunity against thyroid and insulin resistance.

In contrast to our study, Tina Mazaheri et al. showed that there was no obvious association between thyroid autoimmunity and insulin resistance. Only patients with higher Ani TPO antibody titer may experience higher insulin levels. [17]

Although numbers of studies support the positive correlation of autoimmune hypothyroidism with insulin resistance which is in accordance with the present study. WJ Riley et al. in their study illustrated that thyroid disease caused by thyroid autoimmunity occurs frequently in patients with IDDM. And there was also increased frequencies of thyroid microsomal antibodies (TMA) than thyroglobulin antibodies. [29]

R. Kadiyala et al. suggested that a pragmatic approach would be to measure anti-TPOAb and TSH in all diabetic patients at baseline, and then restrict subsequent annual testing to only those patients with T1DM, positive antibodies, or TSH concentration in the upper range of normal to avoid and combat complications related to diabetes mellitus. [30]

The study is conducted in the sub-Himalayan region which is a fringe area and contains a mixed population like Nepalis, Lepchas, Bengalis, Tea Garden Tribes, Rajbongshis, etc. This population is unique with respect to ethnicity as well as disease pattern. No study conducted so far in this population especially for the relationship of ATPO-Ab and insulin resistance. The present study suggests that thyroid assessment in patients suffering from Diabetes mellitus is as necessary as a prediction of insulin resistance, impaired glucose tolerance, and other hyperglycemic complications. In thyroid disorders, this will help to prevent the cardiovascular and atherosclerotic phenomenon which is related to both diabetes mellitus as well as a thyroid disorder.

CONCLUSION

The present study was an Institution-based observational cross-sectional study. It was conducted in adult Hypothyroid patients both male and female aged between (20-55) years attending the outpatient department of North Bengal Medical College and Hospital.

The proposed location of this study is situated at Darjeeling district of West Bengal which is a part of the sub-Himalayan terai region with a mixed population. A high prevalence of thyroid dysfunction is present in this area which is unique in this manner. The endeavor of this study is to substantiate the relationship between Anti-TPO antibody levels and Insulin resistance if any inpatient with hypothyroidism.

The result of the present study shows that anti-TPO-Ab positive subjects had significantly higher mean values of TSH and fasting insulin than anti-TPO-Ab negative subjects and a lower mean value of fT4 than anti-TPO-Ab negative subjects. They also had significantly higher values of IR (HOMA-IR) than anti-TPO-Ab negative subjects.

The observation of the present study also points towards a good correlation between anti-TPO Ab status with other parameters such as FBS, fasting insulin, and TSH. Serum fT4 was inversely correlated with anti-TPO-Ab status. Correlation between anti-TPO-Ab status with HOMA-IR is highly significant with $p<0.0001$ which records a strong association between thyroid autoimmunity with insulin resistance for developing diabetes mellitus.

If alerted early these may help prevent the premature development of metabolic syndrome as well as diabetes in advance.

Limitations

No study is without limitations. The present study has certain limitations which are stated below: -

- Since it was a hospital-based study, the prevalence of thyroid dysfunction may not be applicable to the general population.
- The study has dealt with a relatively small sample size (n=122).
- it was conducted within a short period with a single-window of observation.
- A genetic study will be needed to further establish the present topic.

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