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Original Research Article

A STUDY OF SUBCLINICAL HYPOTHYROIDISM IN PATIENTS OF WESTERN INDIA FOR PREVALANCE OF ANTI THYROID PEROXIDASE ANTIBODY AND ITS ASSOCIATION WITH DYSLIPIDEMIA

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ABSTRACT

Background: In comparison to overt hypothyroidism Subclinical hypothyroidism (SCH) is more common and considered as mild thyroid failure. SCH is also having high probability to convert into overt hypothyroidism. It is well established fact that overt hypothyroidism is associated with dyslipdemia but It is controversial in SCH and there are few Indian studies suggesting correlation between TPO positivity and lipid abnormalities in SCH. Hence, Aim of this study to find the association between dyslipidemia and TPO positivity in SCH patients. **Methods:** 175 cases of subclinical hypothyroidism were recruited fromOPD&IPD department of medicine Mahatma Gandhi Medical College & Hospital Jaipur. After overnight fasting blood samples were drawn in the morning and serum TSH, free thyroxine (FT4), free triiodothyronine (FT3), anti-thyroid peroxidase antibody (TPO-Ab) levels, as well as lipid concentrations were measured. **Results:** Significantly high($p < 0.001$) Cholesterol, LDL, TG level in Anti-TPO (+ ve) group while HDL level was significantly low($p=0.003$) in Anti TPO (+ ve)group. Patients were divided according to levels of TSH. Significantly high(p value< 0.001) levels of Anti-TPO, Cholesterol, LDL was found in patients having TSH levels >10 in comparison to TSH levels ≤ 10. while there were no statistical difference (p value >0.05)in values of TG, VLDL, HDL levels between both groups. **Conclusions:** In SCH patients, dyslipidemia is significantly associated with TPO positivity and it may be associated with levels of TSH, such patients may convert into overt hypothyroidism in future. Hence to prevent adverse outcomes early screening, diagnosis, and treatment of SCH patients are recommended.

Keywords: Total cholesterol level, Thyroid-stimulating hormone, Euthyroidism, Subclinical hypothyroidism,Dyslipidemia, Thyroid antibodies

INTRODUCTION

Hypothyroidism is one of the most common endocrine disorders worldwide **(1)**. The prevalence of hypothyroidism in India is 10-11%. Various studies projections on thyroid disease estimated that about 42 million people in India suffer from thyroid diseases **(2)**. Deficiency of thyroid hormones cause Hypothyroidism, which is one of the most common endocrine disorder nowadays. The disease causes generalized slowing of metabolic processes **(3, 4)**. Higher incidence in women than men **(4)**. Subclinical hypothyroidism refers (SCH) to thyroid hormone deficiency in patients who have very less or no standard clinical features of hypothyroidism **(5)**. In SCH there is increased serum thyroid stimulating hormone (TSH) levels with almost normal thyroxine (T4) and tri iodothyronine (T 3) concentrations **(6)**

Most common cause of SCH is chronic autoimmune thyroiditis associated with Anti TPO (Hashimoto's thyroiditis), whereas others include sub-acute thyroiditis, post-partum thyroiditis, previous hyperthyroidism, in association with other autoimmune diseases, thyroid injury/inflammation due to radiation, surgery, medication and thyroid infiltration **(7)**. The prevalence of SCH is reported to be around 4-10% in the adult population, however this varies with different populations, with more cases occurring in iodine sufficient areas (8-10). The prevalence is even higher in people taking thyroid medications. Around 2-5% of SCH patients are likely to progress to overt hypothyroidism every year **(11, 12)**.

Consequences of Subclinical hypothyroidism include increased risk of cardiovascular disease such as CAD, liver disease, dyslipidemia, and neuropsychiatric symptoms, it may lead to infertility, low birth weight and miscarriages. Treatment of Subclinical hypothyroidism in mildly increased TSH is controversial as reported by many studies no benefit with treatment, on other hand highly increased TSH is often treated by thyroid hormone replacement **(13)**.

Conversion rate of SCH patients with positive TPO antibody into overt hypothyroidism is around 5% per year, it is important to screen and diagnose these patients at risk, and hence, TPO antibody measurement is recommended as an integral part of the investigations done for SCH **(14, 15)**.

Thyroid hormones have an important regulatory effect on lipid metabolism, glucose and BP control. Relationship of Subclinical hypothyroidism and lipid profile is controversial while relationship between subclinical hypothyroidism and an increased risk of cardiovascular disease caused by atherosclerosis is shown in some studies, it is not confirmed in the other studies **(16, 17)**. Due to the role of dyslipidemia as an important risk factor of atherosclerosis, which may appear in subclinical hypothyroidism, There are very few Indian studies on this condition, especially in relation to positive TPO antibody. So our aim in this study is to find out study of prevalence of anti-thyroid peroxidase antibody and correlation between dyslipidemia and TPO antibody in SCH patients.

MATERIAL AND METHODS

Hospital based observational study was conducted in patients of sub clinical hypothyroidism detected in the OPD&IPD of general medicine & Endocrinology department of Mahatma Gandhi Medical College & Hospital Jaipur in 2019. Total 175 subjects of subclinical hypothyroid were included in this study. Each subject measured for lipid profile and presence of Anti TPO antibody. Subjects were divided into 2 groups on the basis of presence or absence of Anti TPO antibody. Each group compared for lipid profile. All Patients measured for FT3 ,FT4, TSH, Anti TPO antibody by CLIA assay and TG, Cholesterol, HDL, LDL,VLDL by spectrophotometric method on Ortho clinical Vitros 5600. Based on inclusion and exclusion criteria, a random selection of subjects for the study was made on basis of detailed history and proper clinical examination. Subclinical hypothyroidism labelled when patient have Normal FT3, FT4 and elevated TSH. Adult patients admitted in IPD and attending OPD without signs of overt hypothyroidism where TSH found to be elevated in routine screening, In Patients with vague symptoms such as easy fatiguability, constipation, depression, mild thyroid enlargement, cold intolerance, Patients with unexplained dyslipidemia and in certain risk groups of sub clinical hypothyroidism like women >35 years, elderly male >65 year were included in this study while Females in gestational or post-partum period, Patients with thyroid destruction(from radioactive iodine or surgery), Patients receiving medications which may cause thyroid dysfunction (eg- amiodarone, lithium & anti thyroid drugs), Patients with end stage renal disease, Post MI, CHF, T2 DM, Patient on any drugs which may interfere in lipid metabolism, Women on OCP, Patients with familial hypercholesterolemia were excluded from this study.

RESULTS

Out of 175 cases of subclinical hypothyroidism 110 cases (62.8%) were TPO -ve and 65 cases (37.14%) were TPO +ve. Mean age in Anti-TPO (- ve) group was 37.90± 13.35 years and Anti-TPO (+ ve) group was 37.20± 13.55.

Table 1: Distribution of cases according to Anti TPO Antibody

Groups	Number of patients	Age (Years)	t value	P value
Anti-TPO (- ve)	110	37.90±13.35	0.333	0.739(NS)
Anti-TPO (+ ve)	65	37.20±13.55		

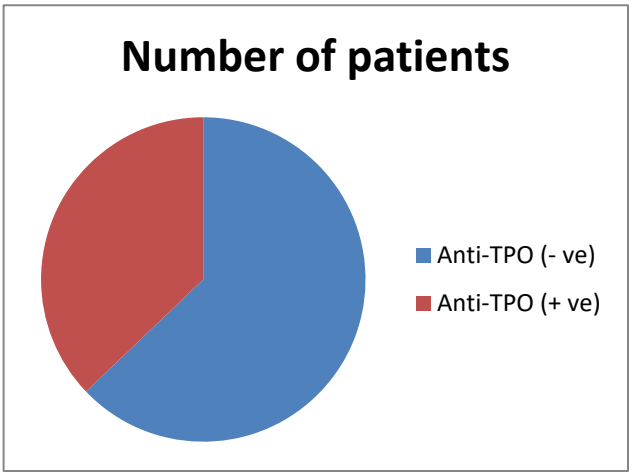

Fig 1 : Distribution of cases according to Anti TPO Antibody

Mean level of Anti-TPO in Anti-TPO (- ve) group 18.07 ± 6.39IU /ml and in Anti-TPO (+ ve) group 278.05± 221.65 IU /ml. This shows significant difference (p value <0.001).between both groups. In this study we found mean FT3,FT4,TSH in Anti-TPO (- ve) group 2.96± 0.54pg/ml, 1.22± 0.37ng/ml and 8.13 ± 5.51µIU /ml respectively and in Anti-TPO (+ ve)group was 2.87± 0.56 pg/ml, 1.19± 0.39 ng/ml and 15.35± 9.81 µIU /ml respectively . No statistically significant difference between both groups in FT3, FT4 levels while TSH levels were significantly high in Anti TPO + ve group (p <0.0001)

Table 2: Comparison of Lipid profile on basis of Anti-TPO levels

Parameters	Anti-TPO (- ve)	Anti-TPO(+ve)	P-value
Anti TPOAb (IU/ml)	18.07±6.39	278±221.65	<0.0001
FT3 (pg/ml)	2.96±0.54	2.87±0.56	0.288 (NS)
FT4 (ng/ml)	1.22±0.37	1.19±0.39	0.612 (NS)
TSH (µIU /ml)	8.13±5.51	15.35±9.81	<0.0001
TG(mg/dl)	143.95±75.59	179±102.58	0.01
Chol. (mg/dl)	158.88±40.45	193.83±49.34	<0.0001
HDL (mg/dl)	43.52±11.73	38.2±10.39	0.003
LDL (mg/dl)	88.45±27.96	119.82±36.69	<0.0001
VLDL (mg/dl)	30.09±17.85	35.81±20.52	0.054 (NS)

In this study significantly high(p < 0.001) Cholesterol, LDL, TG level in Anti-TPO (+ ve) group which was 193.83± 49.34 mg/dl, 119.82± 36.69 mg/dl and 179.0± 102.58 mg/dl respectively in comparison to Anti-TPO (- ve)group 158.88 ± 40.45mg/dl, 88.45 ± 27.96 mg/dl and 143.95 ± 75.59 mg/dl respectively were found while mean HDL level was significantly low(p=0.003) in Anti TPO (+ ve)group which was 38.20± 10.39 mg/dl in Anti TPO + ve group while 43.52 ± 11.73 mg/dl Anti-TPO (- ve) group.

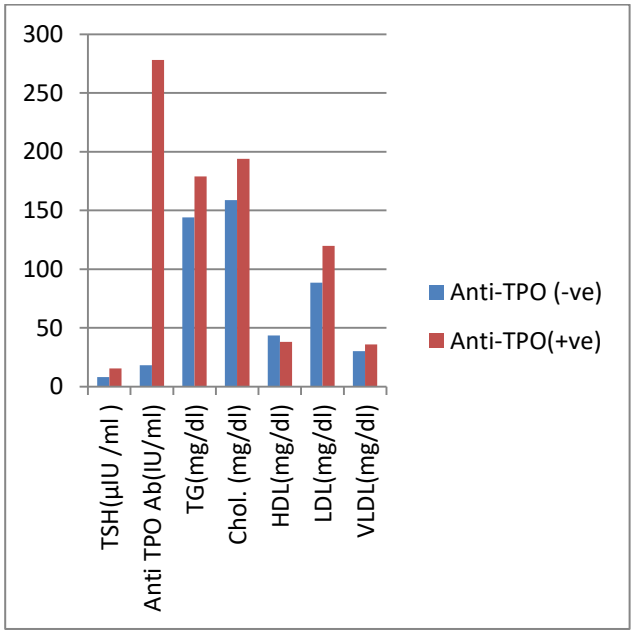

Fig 2: Comparison of Lipid profile on basis of Anti-TPO levels

We divided the patients according to TSH levels into 2 groups TSH ≤ 10, TSHlevels > 10. Total 120 patients had TSH levels ≤ 10 and 55 patients had TSH levels > 10. Mean age for patients who were having TSH levels ≤ 10 was 37.69± 13.05 years and for patients TSH levels > 10 was 37.53± 14.22 years. There were no significant difference of age between both groups (p value 0.942).

Mean FT3, FT4,TSH level in patients having TSH levels ≤ 10 was 2.96± 0.54pg/ml, 1.22± 0.37 ng/ml and 6.81± 1.57 µIU/ml respectively and for TSH levels >10 was 2.84± 0.53 pg/ml, 1.18± 0.38 ng/ml and 19.56± 9.79 µIU/ml respectively. There was no statistical difference between mean FT3 level, FT4 levels according to TSH levels while TSH level show significant (p <0.0001) difference between both groups.

In this study significantly high (p value< 0.001) levels of Anti-TPO was found in patients having TSH levels >10 in comparison to TSH levels ≤ 10. It

was 54.71± 113.30 µIU/ML for TSH levels ≤ 10 and for TSH levels >10 was 245.38± 236.14 IU/ML. Comparison of lipid profile with serum TSH levels we found significantly high (p value <0.0001) cholesterol, LDL levels in patients had TSH levels >10 in comparison to patients had TSH levels ≤ 10. Mean Cholesterol and LDL level in patients having TSH levels ≤ 10 was 166.54± 46.85 mg/dl and 96.10± 39.84 mg/dl respectively and for TSH levels >10 was 196.08± 53.76 mg/dl and 122.43± 41.33 mg/dl respectively. While there were no statistical difference (p value >0.05) in values of TG, VLDL, HDL levels between both groups. Mean TG and VLDL, HDL level in patients having TSH levels ≤ 10 was 150.05± 91.22 mg/dl, 30.32± 18.38 mg/dl and 42.22± 12.21 mg/dl respectively and for TSH levels >10 was 172.13± 77.12 mg/dl, 34.38± 15.78 mg/dl and 40.18± 09.73 mg/dl respectively.

Table 3: Comparison of different parameters on the basis of TSH levels

Parameters	TSH ≤ 10	TSH> 10	P-value
Number of patients	120	55	
Age(years)	37.69±13.05	37.53±14.22	0.942
FT3(pg/ml)	2.96±0.54	2.84±0.53	0.172
FT4(ng/ml)	1.22±0.37	1.18±0.38	0.511
TSH (µIU /ml)	6.81±1.57	19.56±9.79	<0.0001
Anti TPOAb	54.71±113.30	245.38±236.14	<0.0001
TG(mg/dl)	150.05±91.22	172.13±77.12	0.121
Chol. (mg/dl)	166.54±46.85	196.08±53.76	<0.0001
HDL(mg/dl)	42.22±12.21	40.18±9.73	0.277
LDL(mg/dl)	96.1±39.84	122.43±41.33	<0.0001
VLDL (mg/dl)	30.32±18.38	34.38±15.78	0.08

Fig 3: Comparison of Lipid profile and Anti TPO on the basis of TSH levels

DISCUSSION

Thyroid diseases are, undebatably, one of the commonest endocrine disorders in world. India too is not an exception. Subclinical hypothyroidism (SCH) is defined as high Serum TSH concentration with normal serum free Tri-iodothyronine (FT3) and free Thyroxin (FT4) concentrations, allied with few or negligible sign and symptoms of hypothyroidism **(18)**. Generally, it is primary hypothyroidism caused by iodine deficiency, autoimmunity and other reasons. The autoimmune thyroid diseases are associated with number of auto antibodies, which are categorized as either primary or secondary antibodies **(19, 20)**. One of the major secondary antibodies associated with autoimmune thyroid disease are Anti thyroid Peroxidase Antibodies (Anti TPO ab).The most common autoimmune thyroid disease is Hashimoto thyroiditis which is characterized by gradual thyroid failure with or without goitre development **(21)**.

Among general people anti-thyroid antibodies and thyroid profile (TSH, T4, and T3) relation is not established. In several years presence of Anti-TPO antibodies may lead to progress of clinical thyroid disease **(22)**. Determining anti-thyroid antibodies and thyroid profile relation could make out such group of patients who have unstable thyroid profile. Therefore, to rule out underlying autoimmune method screening for Anti-TPO antibodies is required. Diagnosis of autoimmune thyroid disorders is important for treatment modifications for autoimmunity. Studies have shown conflicting results concerning not only the degree of lipid changes in SCH but also the effect of thyroxine substitution therapy. Very few Indian studies are available of lipid profile and SCH. In this study an attempt was made to determine level of anti thyroid peroxidase antibody in subclinical hypothyroidism and establish its association with dyslipidemia.

In this study we included 175 patients of subclinical hypothyroidism as cases out of 175 cases 110 cases (62.8%) were TPO -ve and 65 cases (37.14%) were TPO +ve. Mean age in Anti-TPO (- ve) group was 37.90± 13.35 years and Anti-TPO (+ ve) group was 37.20± 13.55years .It shows in our study both groups were age matched (p value =0.739). Similar study conducted by jusmita et al(23) in 2019 included 30 cases of subclinical hypothyroidism in their study and shows 51.5% cases were Anti - TPO positive while remaining were Anti TPo -ve. Another similar study by Jay shankar CA et al **(24)** in 2015 showed patients with Subclinical Hypothyroidism showed (50%) anti-TPO positivity. In this study many patients were TPO antibody +ve. For cases with negative results for Anti-TPO antibodies, it could be attributed to on-going autoimmune destruction in some of the cases. The continuing on-going active autoimmune process may lead to high antibody titres later. In this study maximum cases had symptoms of lethargy 52% Followed by cold intolerance 21.7%, constipation 20.6%,weight gain 20%, Dry skin 19.4%, Dry brittle hair 14.9%, infertility 8.6%, carple tunnel syndrome 6.9% diastolic hypertension

6.3%, hoarseness of voice5.1%, impotency4.6%, sinus bradycardia 4.6%.

In this study we considered Anti TPO level ≤ 31.5 IU/ml as -ve while > 31.5 IU/ml as positive. Mean level of Anti-TPO in Anti-TPO (- ve) group 18.07 ± 6.39IU /ml and in Anti-TPO (+ ve) group 278.05± 221.65 IU /ml in this study. This shows significant difference (p value <0.001) between both groups.In this study we found that mean TSH in Anti-TPO (- ve) group was 8.13 ± 5.51μIU /ml and Anti-TPO (+ ve)group was 15.35± 9.81 μIU /ml. It shows Anti TPO +ve patients had significantly higher levels of TSH levels.(p value <0.001). A similar study conducted by AtluriSridevi et al(25) in 2018 showed similar results according to their study significantly higher (p value 0.036) TSH in TPO antibody positive group than antibody negative group. Our results also correlates with study conducted in Tehran by AmouzegarAet al **(26)** in 2017 which showed similar to observations and the HUNT Studies **(25)** shows similar results also.

In this study we found significantly high(p value 0.001) Cholesterol, LDL, TG level in Anti-TPO (+ve) group which was 193.83± 49.34 mg/dl, 119.82± 36.69 mg/dl and 179.0± 102.58 mg/dl respectively in comparison to Anti-TPO (-ve) group 158.88 ± 40.45mg/dl, 88.45 ± 27.96 mg/dl and 143.95 ± 75.59 mg/dl respectively .These results was in concordance with Vikas Kumar et al (28) 2017 who found in their study that in TPO positive cases, TC, LDL, and TG levels significantly high. Another similar study by Bandyopadhyayet al **(29)** in 2006 showed similar results which also TC,LDL, and TG levels in TPO positive cases showed statistically significant higher levels.

Mean HDL level in Anti-TPO (- ve) group was 43.52 ± 11.73 mg/dl and in Anti-TPO (+ ve) group was 38.20± 10.39 mg/dl in this study. It shows significantly low (p-value =0.003) level of HDL in Anti-TPO (+ve) patients. A similar Turkish study conducted by Tamer G et al **(30)** in 2011 and Topaloglu O et al **(31)** in 2013 showed similar results according to that TPOAb was negatively correlated with HDL-C levels. A contrast study conducted by Dongmei Kang et al **(32)** in 2015 showed opposite results as they found higher serum TC and HDL-C levels in TPOAb positive patients than TPOAb-negative patients in the middle-aged euthyroid population.

We divided the patients of subclinical hypothyroidism according to TSH levels into 2 groups TSH ≤ 10, TSHlevels > 10. Out of 175 patients 120 patients had TSH levels ≤ 10 and 55 patients had TSH levels > 10.Mean age for patients who were having TSH levels ≤ 10 was 37.69± 13.05years and for patients TSH levels > 10 was 37.53± 14.22years . Both groups were age matched. Mean TSH level in patients TSH levels ≤ 10 was 6.81± 1.57 μIU/ML and for TSH levels >10 was 19.56± 9.79 μIU/ML. In this study we found significantly high (p value 0.001) levels of Anti-TPO level in patients having TSH levels >10 in comparison to TSH levels ≤ 10. It was54.71± 113.30 μIU/ML for TSH levels ≤ 10 and for TSH levels >10 was 245.38± 236.14 IU/ML. This finding could potentially suggest a progressive increase in TSH over a period of time may be risk of developing overt hypothyroidism.

In this study we compare lipid profile with serum TSH levels to determine the effect of TSH levels on lipid profile. We found significantly high (p-value <0.0001) cholesterol, LDL levels in patients had TSH levels >10 in comparison to patients had TSH levels ≤ 10. Mean Cholesterol and LDL level in patients having TSH levels ≤ 10 was 166.54± 46.85 mg/dl and 96.10± 39.84 mg/dl respectively and for TSH levels >10 was 196.08± 53.76 mg/dl and 122.43± 41.33 mg/dl respectively. In this study we found high but non-significant (p value >0.05) values of TG, VLDL levels in patients had TSH levels >10 in comparison to patients had TSH levels ≤ 10. Mean TG and VLDL level in patients having TSH levels ≤ 10 was 150.05± 91.22 mg/dl and 30.32± 18.38 mg/dl respectively and for TSH levels >10 was 172.13± 77.12 mg/dl and 34.38± 15.78 mg/dl respectively. There were no significant difference in HDL levels in both groups. Results of our study correlates study conducted by Prabhakaran J et al(33) in 2018 which showed in their study that TSH levels were positively correlated with cholesterol, LDL and negatively with HDL in patients with Subclinical hypothyroidism (r=0.685, P<0.01 r=0.947, P<0.01 r= -.553, P<0.01 respectively). They found no significant correlation of TSH with triglycerides and VLDL levels. Another study conducted by Xing Wanjia et al(34) in 2012 they found in their study positive linear associations between the serum TSH levels and the log-transformed values of both Total Cholesterol levels (P < 0.01), TG(p<0.05). They did not find any evidence of association between the TSH levels and the log-transformed values of HDL-C and LDL-C.

. In Rotterdam study, it was shown that the incidence of atherosclerosis was even higher in SCH if TPO antibody was positive **(35)**. In a study by McQuadeet

al. they assessed the effects of hypothyroidism (TSH>10 μIU/L), moderate SCH (TSH: 6.1–10 μIU/L), and mild SCH (TSH: 3.1–6.0μIU/L) on different cardiovascular risk factors, CAD prevalence, and also all-cause mortality in patients who were at high risk for CAD. All-cause mortality was found to be higher in both genders in hypothyroid and moderate subclinical hypothyroid patients, but not in mild SCH cases **(36)**. It is observed in above studies that SCH and TPO antibody positivity is associated with atherosclerosis and CAD. Therefore, one of the risk factor is be subclinical hypothyroid **(37)**.

Our study showed high Anti TPO positivity and its significant association with dyslipidemia in SCH patients. All these patients had high risk of developing vascular disease due to endothelial and myocardium dysfunction and overt hypothyroidism due to deranged lipid profile as well as high all-cause mortality in future and so such patients need screening and thorough investigation for early diagnosis and appropriate treatment.

Therefore, all suspected cases of SCH should be screened at primary care level by thyroid profile testing (FT3, FT4, TSH), and if they are found out to be SCH, it must be examined and investigate thoroughly for possible other adverse effects also, associated conditions, and complications of Subclinical hypothyroidism, especially TPO positivity and dyslipidemia.

By early screening at primary care level we can detect SCH patients early and by starting appropriate therapy we can prevent the complications and morbidity such as cardiovascular events and other life threatening complications. Since lack of Indian studies in this field we also recommend large scale, multicenteric studies for better understanding, prevention & treatment of SCH patients.

CONCLUSION

The present study concludes that high prevalence of anti-thyroid peroxidase antibody in subclinical hypothyroidism patients, is suggestive of autoimmune etiology and dyslipidemia is significantly high in such patients. Therefore, it is recommended that early diagnosis is important in subclinical hypothyroidism and will be helpful in preventing adverse outcome,which can be achieved by early initiation of thyroid hormone replacement in such patients.

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