



## Original Research Article

## A study of hs-CRP and lipid profile in hypothyroid adults at tertiary care hospital

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## ABSTRACT

**Introduction:** Thyroid disorders are one of the most prevalent endocrinopathies across the globe. As per the data of India Government, the prevalence of hypothyroidism is about 5.4%. Hypothyroidism is a common endocrine disorder encountered in clinical practice. Hypothyroidism (both clinical & subclinical) has been reported to be associated with inflammation, dyslipidemia which leads to CV risk. Hs-CRP is a marker of chronic subclinical inflammation and a predictor of CVD. The mechanism responsible for inflammatory process seen in hypothyroidism might be TSH induced produced production of TNF- $\alpha$  by a bone marrow cells.

**Aims and Objectives:** To analyse the thyroid profile, hs-CRP and lipid profile in newly detected hypothyroid adults in comparison to controls and also to compare the above parameters in subclinical and clinical hypothyroid cases.

**Materials and Methods:** A total of 164 subjects (82 hypothyroid cases and 82 euthyroid (controls), visiting General Medicine OPD, RRMCH, Bengaluru were consider for study. The subjects were selected based on thyroid profile-analysed by CLIA, hs-CRP by Immunoturbidimetric, Lipid profile was measured by spectrophotometric method. Statistical analysis was done using student's "t" test and Pearson's correlation.

**Result and Discussion:** The study subjects were age matched (mean age 35 years) with female predominance. TSH value significantly increased in cases ( $p < 0.001$ ) however, FT3 & FT4 levels were within the reference range. Serum hs-CRP levels though significantly increased in cases ( $p = 0.005$ ), were within the normal range. There was significant increase in LDL-c and TG ( $p < 0.05$ ) and decrease in HDL-c ( $p < 0.001$ ) in cases significantly. The mean values of serum cholesterol were found to be lower in cases. A positive correlation was found between TSH and hs-CRP ( $r = .275$ ,  $p < .001$ ). ANOVA test showed that the difference in the mean between TSH and hs-CRP was found to be statistically significant ( $p < 0.001$ ).

**Conclusion:** Hypothyroidism (CH & SCH) is common among females and is associated with mild dyslipidemia and low-grade inflammation. Moreover subclinical hypothyroidism is more common than clinical hypothyroidism.

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### 1. Introduction

Thyroid dysfunction is one of the most prevalent endocrinopathies across the globe.<sup>1</sup> The prevalence of spontaneous hypothyroidism is 1-2% of all the thyroid disorders in the world.<sup>2</sup> In India thyroid disorders are the second most common glandular disorder of the

endocrine system and are increasing predominantly among women.<sup>3</sup> Hypothyroidism is characterized by deficient thyroid hormone production which can be severe or moderate.<sup>4</sup> Common etiologies of hypothyroidism are dietary deficiency of iodine and Hashimotos thyroiditis, an auto-immune disease.<sup>5,6</sup> Hypothyroidism is known for its effects on different organs system, leading to hypometabolism. Thyroid gland regulates a wide array of metabolic parameters of carbohydrate and lipid metabolism

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and has profound effects on the cardiovascular system.<sup>7</sup>

Severe deficit of thyroid hormones defines clinical hypothyroidism (CH) and is biochemically characterized by TSH concentration (usually  $>10 \mu\text{IU/L}$ ) with low levels of free thyroxine (FT4) and or Free Triiodothyronine (FT3). The moderate form, called subclinical hypothyroidism (SCH) is defined biochemically as serum TSH concentration above the upper limit of reference range ( $> 4.5 - 10 \mu\text{IU/L}$ ) with thyroid hormone levels that remain within the reference range.<sup>8</sup> CH has been found to be associated with atherogenic dyslipidemia by various researchers and is associated with higher risk of cardiovascular disease. It is known that CH is an established risk factor for atherosclerotic cardiac disease and associated with dyslipidemia and low-grade inflammation and hypercoagulability, but whether SCH is related to risk of CVD is controversial.<sup>9</sup>

The effect of serum thyroid hormones on lipid profile is a complex phenomenon. Thyroid hormones have various effects on both synthesis and degradation of lipids *in vivo*.<sup>10</sup> It acts predominantly through regulation of gene expression related to lipid metabolism.<sup>11</sup> Thyroid hormones through its nuclear receptors, induce HMG- Co enzyme A reductase, which is the first as well as the regulatory step in cholesterol biosynthesis, upregulates low density lipoprotein (LDL-c) receptors by gene activation and maintains serum TG by stimulation of tissue lipoprotein lipase enzyme. It also reduces the plasma HDL by increasing the activity of cholesteryl-ester transfer protein (CETP), hepatic lipase, expression of HDL receptors in the liver and helps reverse cholesterol transport through increased excretion of bile acids in the liver.<sup>12</sup>

Dyslipidemia is a common finding in patients with CH, consisting of high levels of total and LDL-c. Data regarding TG, HDL, Lp(a), apolipoprotein B, A1 components are scarce, reporting either higher or similar to euthyroid subjects levels.<sup>13</sup> The detected abnormalities in the lipid components are significantly improved after thyroxine replacement treatment. However, not all lipid parameters are corrected suggesting a more complex cause of dyslipidemia in hypothyroidism.<sup>14</sup>

According to the Rotterdam study, a serum TSH level  $> 4.0 \mu\text{U/l}$  was found to be associated with a history of myocardial infarction and atherosclerosis of the abdominal aorta.<sup>15</sup> Contradictorily, the Whickham survey found no relationship between initial TSH levels and the development of ischemic heart disease over 20 years of follow up.<sup>16</sup>

Several signs and symptoms with hypothyroidism recommend an abnormality of inflammation. Thyroid hormones play an important role in cardiovascular hemodynamics, yet the association between elevated thyroid hormones and low-grade inflammation is still unknown.<sup>17</sup> The chronic inflammatory state lead to impaired endothelial dysfunction and vascular remodeling.

High sensitive c- reactive protein (hs -CRP) is a marker of chronic subclinical inflammation. Increased hs -CRP levels might be a key molecule linking inflammation to oxidative stress in atherosclerosis (Singh et al) leading to CV risk.<sup>18,19</sup> TSH induced synthesis of TNF- $\alpha$  by bone marrow cells might be the mechanism responsible for inflammatory process seen in hypothyroidism. The other significant finding is major role played by Cyclo-oxygenase-2 (COX 2) which is reported to enhance the production of autocoids that develop inflammation and as a consequence, endothelial dysfunction.<sup>20</sup>

Circulating concentrations of CRP fluctuate widely during acute responses to tissue damage or infection. It is a potential marker of more subtle and persistent systemic alterations that may be known as low grade inflammation.<sup>21</sup> Possible role of CRP in atherogenesis might be due to enhanced expression of local endothelial cell surface adhesion molecules, endothelin-1, reduced endothelial nitric oxide bioactivity. To explore the moderate elevations as in screening, performance of hs-CRP is recommended to better identify CRP variations.<sup>22,23</sup>

In our study we hypothesized that hypothyroidism is associated with mild dyslipidemia associated with chronic inflammatory state as measured by hs-CRP. The basic aim is to study the same in the newly detected hypothyroid adults.

## 2. Objectives

1. To determine the thyroid profile, lipid parameters and high sensitive C- reactive protein in the study subjects
2. To compare the above parameters in hypothyroid patients (cases) and normal individuals (controls)
3. To compare the parameters in Clinical hypothyroidism (CH) and Subclinical hypothyroidism (SCH)

## 3. Materials and Methods

Study design: Hospital based Cross sectional study

Study setting: Department of Biochemistry in collaboration with General medicine, RRMCH, Bengaluru

Study population:

**Group 1: Cases** – 82 newly detected hypothyroid adults, age group: 18-55 years

**Group 2: Controls** – 82 normal healthy adults within same age group

### 3.1. Inclusion criteria

Cases: Newly detected hypothyroid cases between age group of 18-55 years attending General medicine OPD, RRMCH, Bengaluru

Controls: Age and sex matched healthy individuals

### 3.2. Exclusion criteria

1. Subjects who haven't submitted written informed consent
2. Subjects having history of any Medical/ Surgical illness like Cardio vascular disorders, Diabetes Mellitus, kidney failure, Liver disorders and other major chronic illnesses
3. Hypothyroid adults with any other medications or treatments

### 3.3. Method of collection of data

Blood samples were collected with full aseptic precautions after obtaining informed consent. Clot activator that contains vacuum evacuated tubes for analysis of serum TSH, FT3, FT4, TC, HDL-c, LDL-c, TG, hs-CRP. Then after collection, serum samples were stored at -20° until analyzed. Anthropometric measurements for BMI, height (cm) and body weight (kg) were measured without shoes or cap.

### 3.4. Investigations done

1. Serum TSH, FT3 and FT4 by CLIA
2. Serum high sensitive C reactive protein by Immunoturbidimetric assay
3. Lipid parameters analyzed in Erba EM360 autoanalyzer, Serum TG: GPO Method, HDL and LDL cholesterol by precipitation method, Total cholesterol by cholesterol oxidase – peroxidase method.

### 3.5. Statistical analysis

Analysis was done using SPSS version-20 software. The mean and standard deviation for quantitative variables were calculated for the study.

Chi-square test, ANOVA test, students t test were applied whenever necessary. Pearson correlation coefficient was obtained to find out correlation between different parameters. p value < 0.05 was considered to be significant.

## 4. Result

As shown in Table 1, both cases and controls were age matched. The mean age of cases and controls in our study was found to be 35 ± 11 years and 35 ± 10 years respectively (p = 0.76). Approximately 91% of cases and 79% of controls were females depicting a female preponderance (Figure 1). BMI values in the study were higher in cases (26.3 ± 4.8 kg/m<sup>2</sup>) compared to controls (24.8 ± 4.5 kg/m<sup>2</sup>) and was statistically significant (P = 0.04) (Table 1)

In the study, the mean TSH levels (14.26 ± 8.9 μIU/ml) of cases were high compared to controls and was statistically significant (p < 0.001) (Table 2). The mean serum hs -CRP levels in both the study groups was

within the reference range, but it was high and statistically significant in cases than in control (p = 0.005). The total cholesterol level in cases (181.79 ± 41.49 mg/dl) and control (183.22 ± 27.17 mg/dl) were within the reference range and there was no statistical significance (p = 0.79). Further it was found that HDL-c in cases (46.20 ± 8.97 mg/dl) and control (53.0 ± 6.3 mg/dl) were found to be lower in cases compared to controls and the difference was statistically significant (p < 0.001). The mean LDL-c value in cases (144.5 ± 33.9 mg/dl) and control (131.6 ± 31.4 mg/dl) was high in cases and the difference was statistically significant (p = 0.01). The triglyceride levels of cases (158.90 ± 51.64 mg/dl) were significantly higher than that of control (145.65 ± 28.35 mg/dl) and was statistically significant (p = 0.04).

As in Table 3, hs -CRP levels were in within reference range for 78% of cases and 92.7% controls whereas above the normal range was seen in 22 % cases and only 7% controls.

(Table 4) As per the Pearson's correlation, there was a significant positive correlation between serum TSH and hs -CRP levels in cases (r = 0.27, p < 0.001).

To analyse the condition, Hypothyroid cases (n = 82) in our study was divided into two groups (subclinical hypothyroid and clinical hypothyroid) based on TSH and thyroid hormone levels. Out of 82, 65% (n = 53) were subclinical hypothyroid (SCH) and 35% (n = 29) were clinical hypothyroid (CH) cases. A definite female preponderance was observed in the study.

In Table 5, the mean age, BMI between the two groups did not differ significantly. There was a significant increase in serum TSH in CH (23.7 ± 8.7 μIU/ml) as compared to SCH (9.0 ± 2.4 μIU/ml). The difference was statistically significant (p < 0.001). hs -CRP levels though high in CH than SCH were statistically insignificant (p = 0.64). Total cholesterol value was within the reference range in both the groups (CH and SCH) whereas TG was found to be high in CH compared to SCH and was found to be significant (p < 0.001).

There was no significant difference in HDL-c and LDL-c between the two groups (SCH & CH).

TSH and hs- CRP when compared between SCH, CH and controls showed a statistically significant difference between groups with p value <0.001. (Table 6)

**Table 1:** Comparison of cases and controls according to age and BMI

	Cases n=82	Controls n=82	P Value
Age	35.43±11.47	34.93±10.22	0.76
BMI (Kg/m <sup>2</sup> )	26.33 ± 4.89	24.87 ± 4.54	= 0.04*

**Table 2:** Comparison of cases ad controls with biochemical parameters

	Hypothyroid Cases n=82	Controls n=82	P value
T H $\mu$ IU/ml	14.26 $\pm$ 8.9	2.4 $\pm$ 0.96	<0.001*
FT3 pg/ml	1.9 $\pm$ 0.8	2.1 $\pm$ 0.8	= 0.42
FT4 ng/ml	0.8 $\pm$ 0.4	0.8 $\pm$ 0.09	= 1.00
hs-CRP mg/l	4.0 $\pm$ 2.8	2.8 $\pm$ 2.6	= 0.005*
Total Cholesterol (mg/dl)	181.79 $\pm$ 41.49	183.22 $\pm$ 27.17	= 0.79
HDL-c (mg/dl)	46.20 $\pm$ 8.97	53.0 $\pm$ 6.3	< .001*
LDL-c(mg/dl)	144.51 $\pm$ 33.98	131.68 $\pm$ 31.41	= 0.01*
TG (mg/dl)	158.90 $\pm$ 51.64	145.65 $\pm$ 28.35	= 0.04*

**Table 3:** Distribution of cases and controls according to their hs-CRP

hs-CRP mg/l		Hypothyroid Cases =82	Controls n=82
< 5 mg/l		64 (78.0%)	76 (92.7%)
$\geq$ 5 mg/l		18 (22.0%)	6 (7.3%)
Total		82 (100.0%)	82 (100.0%)

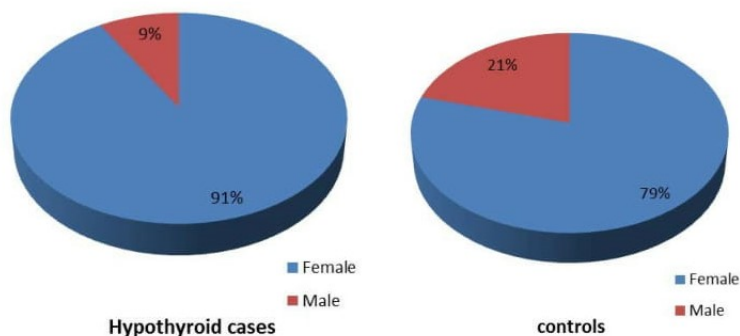
Chi square value =7.029, p value = 0.008

**Table 4:** Pearson’s correlation coefficient between T H vs hs-CRP

Parameters	r value	P value
T H vs hs-CRP	0.275**	<0.001

**Table 5:** Comparison of various arameters among CH and SCH

Parameter	CH =29	SCH n=53	p value
Age (years)	37.72 $\pm$ 12.02	34.17 $\pm$ 11.07	= .18
BMI (kgm2)	26.36 $\pm$ 3.97	26.30 $\pm$ 5.35	= .16
TSH ( $\mu$ IU/ml)	23.7 $\pm$ 8.7	9.0 $\pm$ 2.4	< .001*
FT3 (pg/ml)	1.2 $\pm$ 0.7	2.3 $\pm$ 0.4	< .001*
FT4 (ng/ml)	0.5 $\pm$ .3	1.0 $\pm$ 0.2	< .001*
hs-CRP (mg/l)	4.2 $\pm$ 3.5	3.9 $\pm$ 2.3	= .64
TC (mg/dl)	175.9 $\pm$ 31.1	188.8 $\pm$ 44.9	= .15
HDL-C (mg/dl)	45.5 $\pm$ 9.4	46.5 $\pm$ 8.7	= .61
LDL-C (mg/dl)	148.4 $\pm$ 37.0	142.3 $\pm$ 32.3	= .43
TG (mg/dl)	208.2 $\pm$ 21.6	156.8 $\pm$ 52.9	< .001*



**Fig. 1:**

**Table 6:** Anova of various parameters of SCH, CH and controls

Variables	CH (n=53)	CH (n=29)	Controls (=82)	Total	F value	P value
T H	9.09±2.46	23.70±8.74	1.88± 0.96	8.07±8.86	326.52	< .001
hs- CRP	3.96±2.35	4.23±3.56	2.07±2.6	3.06±2.92	10.63	< .001

## 5. Discussion

Hypothyroidism is by far the most prevalent form of thyroid disorder and is more common in women.<sup>24</sup> It is characterized by a broad clinical spectrum ranging from an asymptomatic/subclinical condition to over the state of myxoedema, end organ effects and multi organ failure.<sup>25</sup> This study has investigated the possible association of hypothyroidism with hs-CRP, lipid profile both reportedly associated with risk of CVD.

A total of 164 subjects participated in this study. Out of the total. 82 subjects were newly detected hypothyroid subjects (cases) and 82 were healthy control. Both the cases and control were age matched. The mean age of cases and control was 35±11 years and 35±10 years respectively (p=0.76) (Table 1). Thyroid dysfunction is a common endocrine disorder with its prevalence increasing with age. About 91% of cases and 79% of control were females showing a female preponderance. Hypothyroidism is known to inflict females more than males (Figure 1). Devika Tayal et al (2012) in their study observed a similar female predominance with a female to male ratio of 2.86 (females 5542 vs Males 1933) A redox imbalance elicited by estrogen could be responsible for increased prevalence in female.<sup>26,27</sup>

In this study BMI was higher in hypothyroid cases. Study conducted by Nivedita Nanda et al (2012) , Kunal B.K<sup>28</sup> et al (2012) reported similar observation with BMI in hypothyroidism. Thyroid hormones mediate their effects mainly through mechanism that stimulate basal metabolic rate, increase ATP expenditure, modulate adrenergic receptor number and responsiveness to catecholamines. Hypothyroid state characterized by slowing down of basal metabolic rate may be an important factor contributing to increase BMI in these cases.

The mean level of serum TSH was significantly higher in cases compared to control (14.26 ± 8.9 vs 2.4 ± 0.96  $\mu$ IU/mL) respectively and was statistically significant (p<0.001). The mean serum FT3 levels (1.9 ± 0.8 vs 2.1 ± 0.8 pg/ml, p = 0.42) and serum FT4 levels (0.8 ± 0.4 vs 0.8 ± 0.09 ng/ml, p = 1.0) in both cases and control respectively were within the normal range (Table 2). Study done by Mohsin Shafi et al (2013) on newly detected hypothyroid patients found that mean TSH levels higher in cases as compared to control (14.3 ± 10.1  $\mu$ IU/L vs 1.8 ±0.7) and was statistically significant (p<0.01).<sup>29</sup> Thyroid

hormone plays a crucial role in regulation of immune system and has the potential to dampen inflammatory cytokines such as INF- $\alpha$ , IL-6, IL-10. Several signs and symptoms suggest that hypothyroidism is an inflammatory state resulting from interaction of IL-6 on TNF and IL-1 leading to increase hs -CRP in this state. Recent studies found that moderate elevations of CRP correlate with future cardiovascular events justifying the use of this test to evaluate cardiovascular risk.<sup>15</sup>

This study showed that mean serum hs -CRP levels in both study groups were within reference range but the mean serum hs-CRP levels in cases was significantly higher (p=0.005) than in control. A significant positive correlation was also found between serum TSH and hs-CRP levels in cases (r = 0.27, p < 0.001) (Table 4).

Christ-crain et al (200 3) observed an elevation in CRP levels with progressive thyroid failure and a clear association between hypothyroidism and increased hs-CRP.<sup>30</sup> Tuzcu et al (2005) , Alpaslan T et al (2005)<sup>15</sup> in their stu dies of the association between coronary heart disease and SCH have reported that elevated hs -CRP levels suggest low grade inflammation predictive of CV risk in hypothyroid subjects.<sup>15</sup>

In contrary to this, a study conducted by Aksoy DY et al (2013) on women could not validate a significant difference in hs-CRP levels between hypothyroid and control.<sup>31</sup>

The interaction of IL-6 on TNF- $\alpha$  and IL-1 results in the raised CRP levels in hypothyroidism. Lack of thyroid hormones may impair the rate of CRP clearance which may be one reason in increase in serum CRP level. Similarly, slow CRP uptake in target cells might also add to this phenomenon. The low grade inflammation which may be accountable for increased risk of developing CVD in hypothyroidism.<sup>17</sup>

Thyroid disorders are known to influence lipid metabolism and other CV risk factors predominantly. Dyslipidaemia is a well-recognized association of thyroid dysfunction which should be considered in the process of evaluating and treating dyslipidemic patients.<sup>32,33</sup>

In this study, it was found that total cholesterol values had no statistical significance but HDL-c in cases was found to be lower compared to control and the difference was statistically significant (p < 0.001). The mean LDL-c and triglyceride in cases were higher than control with p = 0.001 and p = 0.04 respectively.

Sunanda et al (2012) found that there was a strong positive association between TSH and lipid profile in hypothyroid patients and concluded that effect of

hypothyroidism on the serum lipids is more pertinent in patients with higher TSH levels.<sup>34</sup> Khan Mah et al (2013) also found a significant dyslipidemia i.e. significant increase in TC, LDL-c and TG levels and decrease in HDL-c levels.<sup>35</sup>

Slight elevation in TSH levels, preponderance of sub-clinical hypothyroid subjects and shorter duration of illness (newly detected cases) might be likelihood causes of mild dyslipidemia observed in the study.

In clinical hypothyroidism (CH), a decrease in LPL activity and the clearance of TG-rich lipoproteins<sup>36</sup> are found. Therefore CH patients may also present with elevated TG levels associated with increased levels of VLDL and occasionally fasting chylomicronemia<sup>37</sup> as observed with hypertriglyceridemia in hypothyroid cases in the study. Many previous studies concluded that CH patients have elevated atherogenic and oxidative stress markers. Hence, serum TSH measurement is the essential test for diagnosis of mild thyroid failure when the peripheral thyroid hormone levels are within normal reference range.<sup>38</sup>

In this study, a decreased HDL levels in cases was found. Clinical studies however reported a conflicting result about HDL-cholesterol plasma levels in hypothyroidism. The studies conducted by Caron et al found a reduction in HDL cholesterol and an increase in HDL after subsequent treatment with thyroxine. However, S. Valdemarsson et al and E. Muls et al found an improvement in the mean HDL levels in the hypothyroidism with a reduction after treatment. Several proteins related with HDL metabolism are affected by thyroid hormones.<sup>39–41</sup>

The extent to which various levels of thyroid dysfunction affects CV event need to be debated.<sup>22</sup> Cappola et al in their study of Cardiovascular Health Study data found that there was no relationship between SCH or CH and prevalence of atherosclerotic disease, cardiovascular mortality or all causes mortality.<sup>42</sup>

For better analysis in this study, hypothyroid cases were divided into subclinical hypothyroid (SCH) and clinical hypothyroid (CH). Majority of the target study group was female. The mean age, BMI values between two groups did not differ significantly. The CH subjects has higher serum TSH levels as compared to the subjects of SCH which was statistically significant ( $P < 0.001$ ). hs -CRP levels were found to be at risk level and was comparable in both CH and SCH but was statistically insignificant ( $p = 0.64$ ). Many studies have shown that high levels hs-CRP in women with SCH correlated with parameters of obesity which emphasizes the role of body weight in inflammation<sup>43</sup> and may consider as an additional risk factor for the development of atherosclerosis and CVD.<sup>44–46</sup>

Total cholesterol value was found to be within reference range in both the groups (CH and SCH) but TG value was found to be high in CH as compared to SCH which was found to be statistically significant ( $p < 0.001$ ). There was

no significant differences in HDL-c, LDL-c between two groups. Cross sectional studies conducted by Jung CH et al (2003) also reported that SCH is associated with increased levels of TC and LDL-c.

Thus subjects with CH in this study resulted in hypertriglyceridemia but in SCH subjects, other than the mild degree of TSH elevation and slight increase triglycerides, there was not any significant changes in the parameters studied.

The mean values of serum TSH and hs-CRP were higher in CH as compared to SCH and controls. It was observed that the difference was statistically significant ( $p < 0.001$ ).

Studies conducted by Biondi and Cooper have shown that SCH is associated with variable and inconsistent changes in above mentioned parameters in contradictory to CH in which significant changes are normally found.<sup>47</sup>

Two large population based studies-Whickham survey, National Health & Nutrition Examination Survey III (NHANES III) and other have highlighted the fact that serum TSH is the most powerful predictor of the outcome of SCH over a period of time. TSH values  $> 4 \mu\text{IU/L}$  and the presence of thyroid auto antibodies had very high chances of progression to overt hypothyroidism.<sup>48,49</sup>

Hence, hypothyroid cases should be investigated for dyslipidemia and inflammatory markers since they help in prediction of CV risk. It is also essential to assess the progression to the clinical state and to finalize the course of treatment.

## 6. Limitations of the study

Small sample size and cross sectional study.

## 7. Conclusion

This study demonstrates that hypothyroidism is associated with dyslipidemia and low grade inflammation. Subclinical hypothyroidism was found to be more common than clinical hypothyroidism. Hypertriglyceridemia and at risk hs -CRP levels though seen in hypothyroid cases were more prominent in CH cases than SCH. The mild and inconsistent changes which were observed in the biochemical parameters in hypothyroidism (i.e. combination of both CH and SCH) may be due to the preponderance of subclinical hypothyroid cases in this study. However, dyslipidemia and inflammatory markers were found to be increased in the cases that helped in prediction and evaluation of patients at risk of cardiovascular disease.

## 8. Source of funding

None.

## 9. Conflict of interest

None.

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