

# Association of Elevated Serum hs-CRP Levels with the Development of Cardiovascular Disease in Known Cases of Hypothyroidism: A Case-control Study

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## ABSTRACT

**Introduction:** Hypothyroidism is linked with an increased risk of Cardiovascular Diseases (CVD) and associated risk factors, but there is little documented information available about its association with high-sensitivity C-Reactive Protein (hs-CRP).

**Aim:** To analyse the association between hs-CRP and CVD in hypothyroidism.

**Materials and Methods:** This case-control study was carried out among 159 participants (87 cases and 72 controls) from January 2015 to August 2016 in the Department of Biochemistry, in collaboration with Department of Medicine, Subharti Medical College, its associated Chatrapati Shivaji Subharti (CSS) Hospital, Meerut, Uttar Pradesh, India. The samples were divided into two groups - cases (hypothyroid patients) and control group. Blood sample (3 mL) was collected from each subject. Serum was separated from blood by centrifuging blood at 3000 rpm for 10 minutes. Estimation of hs-CRP was done on semi-autoanalyser (Robonikreadwell touch) in clinical biochemistry laboratory. Lipid profile and other routine investigations were

done by Vitros-250 autoanalyser Johnson & Johnson, USA. All the statistical analysis was done by using the Windows based Statistical Package for Social Sciences (SPSS) version 18.0. Student's t-test and Pearson correlation analysis was applied and p-values <0.05 were taken as the level of significance.

**Results:** The mean age of cases and controls were 47.50±7.9 and 41.66±9.3 respectively. The mean hs-CRP values in the subjects with hypothyroidism (cases) was 3.41 mg/L and in control was 2.10 mg/L (p-value<0.0001). The weight and waist circumference, were also significantly different (p-value<0.05) between the study groups. The hs-CRP had an insignificant correlation with the BMI (r= 0.12, p=0.23) as well as with waist circumference (r=0.15, p=0.14). Non significant correlation was found in between the hs-CRP and the blood pressure.

**Conclusion:** This study concludes that hs-CRP levels were significantly higher in patients with hypothyroidism when compared to the control group. A significant correlation was found between cardiovascular risk factors like total cholesterol and HDL and hs-CRP levels.

**Keywords:** Complication, Serum lipid, Waist circumference

## INTRODUCTION

The hs-CRP is an extremely sensitive marker of inflammation, and it is among the several biomarkers that have been proposed for Cardiovascular Diseases (CVD) progression [1,2]. The hs-CRP contributes to the recognition of people at risk of developing CVD in the near future [3-6]. Due to inflammation there are marked vascular changes that can rarely be evaluated using regular cardiac imaging methods. So, the role of estimation of various inflammatory biomarkers in peripheral blood has increased considerably with time, with hs-CRP being the most thoroughly studied in CVD [7,8]. Estimation of hs-CRP is rapid, simple and quite cheap; also it remains stable in the sample for considerable period of time [7]. Numerous prospective cohort studies have documented the correlation between raised level of hs-CRP and extended CVD risk in subjects either with well-established disease as well as in the development of CVD [2,5,7,9].

Thyroid hormones and Thyroid Stimulating Hormone (TSH) plays a critical role in the normal physiological function of heart and in hypothyroidism their role is directly related to outcomes in CVD. Thyroid hormones and TSH are also associated with the known risk factors of CVD in subclinical and diagnosed CVD cases [10,11].

It makes hs-CRP testing valuable in the known cases of hypothyroidism as a tool of estimation of disease progression and prognosis and also to establish the risk for the development of CVD in hypothyroid patient.

Various studies have documented the association between hs-CRP and CVD [2,7], but the various studies have documented the association between hs-CRP and CVD, but the data on the population residing in western U.P. region of study is very limited. So, the present study was planned to find the correlation of hs-CRP, hypothyroidism and CVD in the population of western U.P. region.

## MATERIALS AND METHODS

This case-control observational study was carried out in the Department of Biochemistry, in collaboration with Department of Medicine, Subharti Medical College, its associated Chatrapati Shivaji Subharti (CSS) Hospital, Meerut, Uttar Pradesh, India, among 159 participants, over a period of 20 months from January 2015 to August 2016. The Institutional Ethics Committee approved the study (letter no. SMC/EC/2015/026) and after that informed consent was taken from all the patients. This study is a part of a broader study on thyroid dysfunction [12]. The sample size of 159 participants constituted 87 patients of hypothyroidism (cases) & 72 control (euthyroid).

The study samples were divided into two groups: (i) cases (hypothyroid patient after having laboratory investigations of T3, T4 and TSH), and (ii) control group (patient attending medicine Outpatient Department (OPD) not having sign and symptoms of CVD and also have diagnosed euthyroid after laboratory investigations of T3, T4 and TSH.)

### Inclusion criteria

For cases: All patients between the age group of 20 and 60 years attending Medicine OPD having signs and symptoms of CVD and hypothyroidism (based on laboratory investigation of T3, T4 and TSH), patients having dyslipidaemia were taken as cases.

For controls: All the patient attending medicine OPD with no sign and symptoms of CVD, euthyroid participants (patients having normal thyroid profile based on laboratory investigation of T3, T4 and TSH), study participants between the age group of 20 and 60 years were included as controls.

### Exclusion criteria

Patients with history of CVD, drugs (effecting CVD) intake one month prior to sampling, on steroids and/or immunosuppressant drugs and patients with Chronic Renal Failure (CRF) were excluded from the study.

### Data Collection

Information about the subject's age, sex, monthly income, lifestyle, family history of diabetes mellitus and other chronic disorders were recorded. Anthropometric measurements like height, weight and waist circumferences were also measured. Body Mass Index (BMI) was calculated as by dividing weight in kg by the square of height in meters. Blood pressure was measured with special precaution.

### Assay Methods

Blood sample (3 mL) was collected from each subject. Serum was separated from the blood by centrifuging blood at 3000 rpm for 10 minutes. Estimation of hs-CRP was done on semi-autoanalyser (Robonikreadwell touch) in clinical biochemistry laboratory. Lipid profile was estimated- Total Cholesterol (TC), Triglyceride (TAG) by enzymatic method and High-Density Lipoprotein (HDL) measured by non HDL precipitation method followed by enzymatic method in serum samples [13]. The low-density lipoprotein (LDL) cholesterol concentration was calculated by friedwald's formula using (TC, HDL, TAG) (LDL cholesterol= TC-HDL-TAG/5 (mg/dL) [14] by Vitros-250 auto analyser Johnson & Johnson, USA.

A turbidimetric immunoassay for the determination of hs-CRP in serum was used. Measuring range is 0.15 mg/L to 5 mg/L [9].

The study followed the risk stratification as recommended by American Heart Association (AHA) [9]:

Low risk: <1.0 mg/L

Average risk: 1.0 to 3.0 mg/L

High risk: >3.0 mg/L

## STATISTICAL ANALYSIS

All the analysis was done by using the Windows based Statistical Package for Social Sciences (SPSS) version 18.0. To evaluate the significance of difference between the groups, Student's t-test was applied between them. Mean±SD (Standard Deviation) was used to present the results, Pearson's correlation analysis was carried out to find the association between the groups and p-value <0.05 was taken as the level of significance.

## RESULTS

In comparison with control subjects, the hypothyroid cases in the study were older (p-value<0.0001) and had higher BMI (p-value=0.01). They also had higher Diastolic Blood Pressure (DBP) (p-value<0.0001). The mean hs-CRP value in the subjects with hypothyroidism was 3.41 mg/L and in control subjects, it was 2.10 mg/L. The hs-CRP levels were significantly higher among the hypothyroid subjects (p-value<0.0001). Weight and waist circumference were also statistically significant between the study groups [Table/Fig-1].

Parameters	Cases (n=87)	Control (n=72)	p-value	t-test
Age (years)	47.50±7.9	41.66±9.3	p<0.0001*	4.21
Height (cm)	149.05±15.1	150.06±17.0	p=0.69	0.39
Weight (kg)	69.88±12.7	63.88±12.94	p=0.0039*	2.96
Body mass Index (kg/m <sup>2</sup> )	32.11±7.41	29.19±8.04	p=0.01*	2.35
WC (Inches)	102.49±9.37	90.47±8.64	p<0.0001*	8.39
Systolic BP (mm Hg)	118.36±11.73	126.23 ± 8.10	p<0.0001*	4.98
Diastolic BP (mm Hg)	87.26±9.76	76.55 ± 2.81	p<0.0001*	10.21
hs-CRP (mg/L)	3.41±0.92	2.10±0.86	p<0.0001*	9.21

**[Table/Fig-1]:** Comparison of characteristics. p-value <0.05 considered significant; \*, Significant; WC: Waist circumference

The correlation of hs-CRP with various anthropometric measurements (Height, weight, BMI, Waist circumference) and with blood pressure (Systolic & Diastolic) was found insignificant with p-value of >0.05 [Table/Fig-2].

	Parameters	r-value	p-value
hs-CRP versus Anthropometric measurement	Age (years)	0.03	0.73
	Height (cm)	-0.15	0.16
	Weight (kg)	0.01	0.91
	Body Mass Index (kg/m <sup>2</sup> )	0.12	0.23
	WC (Inches)	0.15	0.14
	Systolic BP (mmHg)	0.12	0.11
	Diastolic BP (mmHg)	-0.09	0.37

**[Table/Fig-2]:** Correlation factors of serum hs-CRP with the anthropometric measurement in patients of hypothyroidism. p-value<0.05 considered significant

In this study, there was a positive and significant correlation between hs-CRP and TC and HDL [Table/Fig-3].

	Parameters	r-value	p-value
hs-CRP versus Cardiovascular risk factors	Total cholesterol (mg/dL)	0.15	0.04*
	TAG (mg/dL)	0.08	0.43
	HDL (mg/dL)	0.10	0.03*
	LDL (mg/dL)	0.12	0.25
	VLDL (mg/dL)	0.09	0.40

**[Table/Fig-3]:** Correlation factors of serum hs-CRP with the lipid profile as a cardiovascular risk factors in patients of hypothyroidism. Correlation coefficient (r); \*, significance; TAG: Triglyceride; HDL: High density lipoprotein; LDL: Low density lipoprotein; VLDL: Very low density lipoprotein

## DISCUSSION

Hypothyroidism is one of the most prevalent endocrine disorder and the disease burden related to this cluster of disorders is commonly associated with CVD [11,15]. Epidemiological data suggests that not solely subclinical or treated thyroid disease is related to increase cardiovascular risk, however, conjointly completely different varieties of thyroid diseases correlate with increased long term vascular risk [16]. This study also has similar findings that thyroid disorders (hypothyroidism) have significant correlation with cardiovascular risk factors (TC).

In this study, it was found that the hs-CRP values were significantly higher in cases (hypothyroid patient) as compared to controls (euthyroidism). This was in accordance with the study conducted by Christ-Crain M et al., [17]. A study conducted by Tuzcu A et al., found that the mean value of hs-CRP (3.41) thyroid dysfunction patients was 4.2±0.8 mg/L against 1.05± -0.3 mg/L in control group [18]. This was also in accordance with the present study in which there were higher values of hs-CRP in thyroid dysfunction patients. A study

conducted by Sharma R et al., also concluded that subjects with thyroid disorders had significantly higher levels of serum hs-CRP, when compared to healthy euthyroid controls. They also found a significant positive correlation of TC (risk factor for CVD) in hypothyroid patients in their study which is in accordance in the study also [19].

Another case control study documented by Czarnywojtek A et al., found the mean values of hs-CRP in hypothyroid cases to be higher as compared to euthyroid healthy control patients [20].

A cross-sectional investigation of a cohort of 2,494 participants enlisted in Taiwan from year 2006 to 2008 showed an association between hypothyroidism (TSH >5.6  $\mu\text{IU/mL}$ ) and elevated level of hs-CRP, i.e., results are consistent with this study [21]. A clinical trial reported that hs-CRP values elevated more and more with hypothyroidism in 63 subjects with subclinical hypothyroidism ( $p=0.022$ ), and particularly in 61 subjects with overt hypothyroidism ( $p=0.016$ ), in comparison to 40 euthyroid matched healthy controls [17].

The underlying cause of CRP elevation in thyroid disorders is still unclear. But various signs and symptoms in hypothyroid patients indicate an abnormality of inflammation. These are believed to be the result of an interaction of Interleukin-6 on tumour necrosis factor- $\alpha$  and Interleukin-1. This interaction results from the elevated CRP level in hypothyroidism. Aside from the referenced cytokines, a drop in the levels of thyroid hormones results in the slowing down of the general metabolic rate, and all the biochemical processes may be impaired under those circumstances. Thus, the decreased rate of clearance of CRP may result in serum CRP level increase. Similarly, slow CRP uptake in target cells might also add to this mechanism. In contrast, hyperthyroidism results in increase of general metabolic activity, which may lead to the hyperactivity of adrenergic nervous system, stimulation of immune system and remarked increased peripheral blood flow. Each one of these conditions might result in an increase of CRP concentration [20].

Mean BMI in this study cases was found to be 32.11 kg/m<sup>2</sup>. Diaz-Olmos R et al., in their study found mean BMI in their patients to be 28.0 kg/m. Hypothyroid patients have raised BMI and fall under this category [22].

In the study, it was found that Systolic Blood Pressure (SBP) in cases were 118.36 (11.73) mmHg and Diastolic Blood Pressure (DBP) was 87.26 $\pm$ (9.76) mmHg. Near similar values were also reported by Diaz-Olmos R et al., where they found SBP equal to 120.6 (13.5) mmHg and mean DBP equal to 81.0 (8.1) mmHg [22].

Czarnywojtek A et al., Yu YT et al., documented in their studies that patients with hypothyroidism had higher levels of serum hs CRP as compared to control group [20,21]. Similar result was found in this study also i.e., hs-CRP level is found raised in the patients suffering from hypothyroidism, Rajendra KC et al., in their study found that patients suffering from hypothyroidism have positive correlation with risk factor for CVD (TC) and also have raised levels of hs-CRP which is also the findings of this study [23]. Similar inference was drawn in other studies also [17,19].

These patients should be advised for regular monitoring of their thyroid profile so that if there is any derangement occurs, it should be corrected with dose adjustment and inflammatory/cardiovascular changes can be avoided in the near future.

## Limitation(s)

The limited sample size of the study although provided a good insight, a further more insight into the correlation of hypothyroidism and CVD can be drawn from a larger sample size.

## CONCLUSION(S)

High-sensitivity C-Reactive Protein levels were significantly higher in patients with hypothyroidism when compared to the control group. A significant correlation was found between cardiovascular risk factors like total cholesterol and HDL and hs-CRP levels. Present study findings suggest that deranged thyroid hormone levels and TSH regulate the amount of hs-CRP that could lead on to varied complication like CVD.

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