

Study Of Cystatin C Level In Subclinical Hypothyroid Patients

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Abstract- Subclinical hypothyroidism is a common biochemical finding in the general population. It is accidentally diagnosed and usually presents with mild or no symptoms of hypothyroidism, but it may progress to overt hypothyroidism. Patients with hypothyroidism are prone to develop cardiovascular disease, which is related to elevated cholesterol and lipoprotein levels in these patients. Cystatin C is a non-glycosylated neuroendocrine protein, affected by the thyroid state. So this study was conducted with the aim to measure serum levels of cystatin C in patients with Subclinical Hypothyroidism and to find out possible relationship between them. In this study 70 cases of subclinical hypothyroid patients along with 70 healthy subjects (age and gender matched) were enrolled. Cystatin C levels were assessed. Serum Cystatin C levels were decreased in Subclinical Hypothyroid subjects ($P < 0.0001$) when compared to healthy controls. Study concluded that serum cystatin C is a valuable marker in subclinical hypothyroid subjects to predict the possibility of development of any cardiovascular complications accompanying Hypothyroidism.

Keywords: Cystatin C, Hypothyroidism, Subclinical Hypothyroidism.

I. INTRODUCTION

Subclinical hypothyroidism, also known as mild thyroid failure, is defined as an elevated serum thyroid stimulating hormone (TSH) level with a normal serum thyroxine (FT4) concentration.⁽¹⁾ Subclinical hypothyroidism is accidentally diagnosed and usually presents with mild or no symptoms of hypothyroidism, but it may progress to overt hypothyroidism. Thyroid hormones, Thyroxine (T4), and Triiodothyronine (T3) play an important role in all major metabolic pathways by regulating protein, carbohydrate, and lipid metabolism; including synthesis, mobilization, and degradation. Patients with hypothyroidism are prone to develop cardiovascular disease, which is in accordance with autopsy studies showing that the atherosclerotic process is increased in hypothyroidism⁽²⁾ and decreased in hyperthyroidism.⁽³⁾

The impact of Subclinical Hypothyroidism on the cardiovascular system has recently become an important topic of research. Increased incidence of cardiovascular diseases in subclinical hypothyroidism cannot be fully explained by an atherogenic lipid profile. Thyroid hormones are physiologic modulators of both tissue oxidative stress and protein degradation. Oxidative stress increases the concentration of oxidised Low-Density Lipoprotein (LDL).

Dysfunction of thyroid hormones also causes significant variations in kidney function. Hypothyroidism and hyperthyroidism both affect glomerular filtration rate

(GFR), tubular function, renal blood flow, and structure of the kidney. Hyperthyroidism increases GFR due to diminishing the peripheral vascular resistance and increasing the effective renal blood flow and vasodilatation of the renal blood vessels. Conversely, hypothyroidism diminishes the GFR by enhancing the peripheral vascular resistance and diminishing the effective renal blood flow and vasoconstriction of the renal blood vessels.

Cystatin C, a protein encoded by the CST3 gene, is mainly used as a biomarker of kidney function. It protects host tissue from destructive proteolysis by inhibiting the cysteine proteinases.⁽⁴⁾ Recently, it has been studied for its role in predicting new onset or deteriorating cardiovascular disease. It has a low molecular weight, and is removed from the bloodstream by glomerular filtration in the kidneys. If kidney function and glomerular filtration rate decline, the blood levels of Cystatin C rise. Serum levels of cystatin C are a more precise test of kidney function (GFR) than serum creatinine levels. Cystatin C levels are less dependent on age, sex, race and muscle mass compared to creatinine.

Several studies have found that increased levels of Cystatin C are associated with the risk of death, several types of cardiovascular disease (including myocardial infarction, stroke, heart failure, peripheral arterial disease and metabolic syndrome) and healthy aging. Some studies have found cystatin C to be better in this regard than serum creatinine or creatinine-based GFR equations. Because the association of cystatin C with long term outcomes has appeared stronger than what could be expected for GFR, it

has been hypothesized that cystatin C might also be linked to mortality in a way independent of kidney function. In keeping with its housekeeping gene properties, it has been suggested that cystatin C might be influenced by the basal metabolic rate. It was found that Cystatin C levels are affected by the thyroid state, they are increased in hyperthyroidism and decreased in hypothyroidism. (5) Thyroid function affects cystatin C level through changes in the synthesis of cystatin C and its clearance. (6) Some authors reported that cystatin C levels should be measured with TSH to exclude primary thyroid disorders. (7)

Cystatin C levels are decreased in atherosclerotic and aneurysmal lesions of the aorta. Breakdown of parts of the vessel wall in these conditions is thought to result from an imbalance between proteinases and their inhibitors. Cystatin C can reflect the severity of coronary artery disease to a certain extent. Lot of studies have shown significant difference in Cystatin C levels in hyperthyroid subjects while only few studies have been done on hypothyroid subjects especially subclinical ones. The present study was undertaken to find possible difference in Cystatin C level among subclinical hypothyroid subjects and healthy subjects. Secondly, Subclinical hypothyroidism is closely related to hyperlipidemia; Cystatin C concentration is also associated with atherosclerotic lesions which are consequences of hyperlipidemia. Hence, the study also tries to find out the relationship between Cystatin C concentration and subclinical Hypothyroidism.

II. MATERIAL & METHOD

The study was conducted on 70 cases of subclinical hypothyroid patients of both gender and age attending the OPD of Department of Medicine, J.L.N. Medical College & Associated group of Hospitals, Ajmer. Diagnosis of thyroid disorder has been made according to the criteria recommended by the European Thyroid Association Guidelines-2013. The results were compared with age and gender matched 70 subjects acting as controls. Consent from all the subjects was obtained for the study. Blood samples were collected from anticubital vein by venepuncture in plain vials. Serum was separated by centrifugation at 2500 rpm for 10 minutes and stored at - 20⁰ C.

Exclusion criteria- Following cases were excluded:

1. History of renal disease, cerebrovascular or cardiovascular diseases
2. Thyroid supplementation and anti thyroid agents
3. Diabetes Mellitus
4. Chronic Alcoholism
5. Hypertension
6. Pregnancy
7. Positive clinical history of any Malignancy

Serum Free Triiodothyronine (FT₃), Serum Free Thyroxine (FT₄) and Serum Thyroid Stimulating Hormone (TSH) were measured using Enzyme Linked Immunosorbent Assay (ELISA). Serum Cystatin C was also measured using the Enzyme Linked Immunosorbent Assay (ELISA) technique.

Data was recorded in a predesigned performa, managed in an excel spread sheet and was reported as mean

± SD median (range). Comparison of physical and biochemical parameters between Subclinical hypothyroid subjects and Healthy controls was performed using equal or unequal variance unpaired student t-test for continuous variables (as applicable).

All p-values were based on a two sided test of statistical significance. Significance was accepted at the level of p<0.05.

III. RESULTS AND OBSERVATION

In this study, the healthy controls were in GroupI and subclinical hypothyroid subjects in GroupII.

TABLE I: COMPARISION OF BIOCHEMICAL PARAMETERS IN HEALTHY CONTROLS AND SUBCLINICAL HYPOTHYROID SUBJECTS

Parameter	(GroupI) Healthy controls (n=70) Mean±SD	(GroupII) Subclinical Hypothyro id subjects (n=70) Mean±SD	'P' Value*
ft3 (pg/ml)	3.10±0.42	3.02±0.68	P > 0.05
ft4 (ng/dl)	1.56±0.38	1.39±0.56	P < 0.01
TSH (µIU/ml)	2.15±1.09	9.81±2.47	P < 0.0001
Cystatin C (mg/L)	0.85±0.15	0.73±0.19	P < 0.0001

P value <0.0001 is considered Highly Significant (HS), p<0.01 is Significant(S) while p>0.05 is Non Significant (NS)

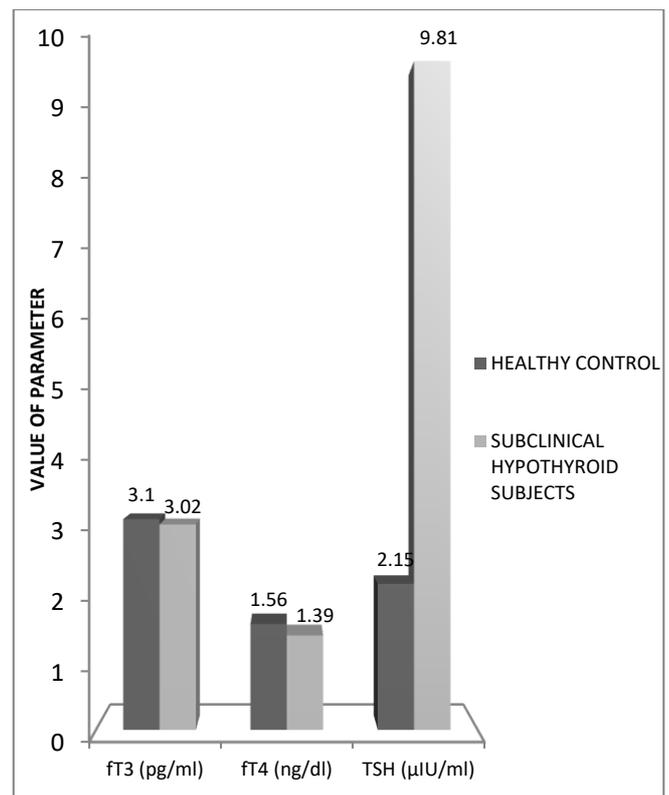


Fig.1 COMPARISION OF ft3, ft4 AND TSH LEVELS IN HEALTHY CONTROL AND SUBCLINICAL HYPOTHYROID SUBJECTS

Table I shows that the mean Serum ft3 level was found to be slightly decreased in Subclinical hypothyroid subjects (group II) as compared to healthy controls (group I) but the difference was not statistically significant. The mean Serum ft4 level was found to be decreased in subclinical hypothyroid subjects as compared to healthy control and the difference was statistically significant ($p < 0.01$) while a highly significant increase in mean Serum TSH level was observed in subclinical hypothyroid subjects (group-II) when compared to controls (group-I).

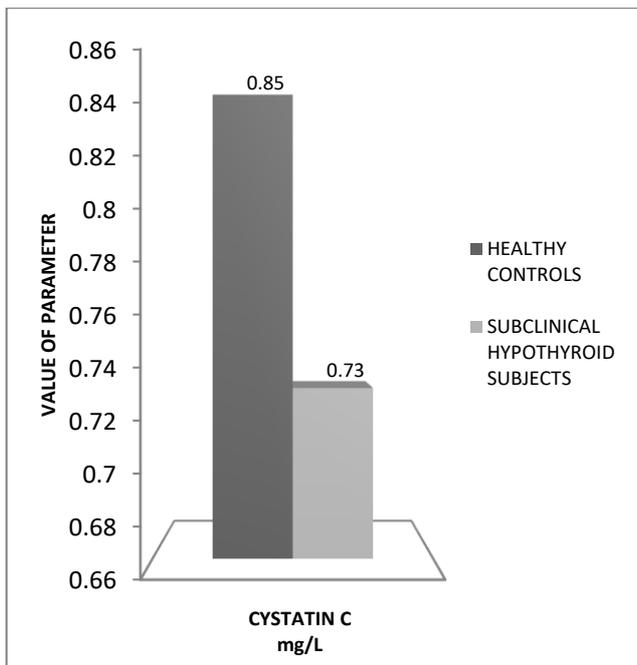


Fig.2 COMPARISION OF CYSTATIN C LEVELS IN HEALTHY CONTROL AND SUBCLINICAL HYPOTHYROID SUBJECTS

In subclinical hypothyroid subjects, serum Cystatin C level was found to be low in comparison to healthy controls, and the difference was highly significant i.e. 0.85 ± 0.15 vs. 0.73 ± 0.19 (Fig2).

IV. DISCUSSION

In recent years, subclinical hypothyroidism is unknowingly emerging as a major public health problem in India and it produces an enormous burden on the economy of the country as it can lead to adverse cardiovascular consequences. Many studies have shown that Cystatin C levels are affected in hyperthyroidism. Very few studies have evaluated Cystatin C levels in subclinical hypothyroidism. The present study was carried out to analyse the role of Cystatin C as a predictive marker for cardiovascular diseases in subclinical hypothyroid cases.

Based on Table I, the mean serum Cystatin C level in subclinical hypothyroid subjects (0.73 ± 0.19 mg/L) was found to be lower than healthy controls (0.85 ± 0.15 mg/L; P

< 0.0001). Our study is in line with Ye et al. (2013) who revealed that changes in Thyroid Hormones can cause a change in Cystatin C levels. Analyzing 520 patients, Yu Weiguo (2016) found that in patients with hyperthyroidism Cystatin C is significantly increased and in hypothyroidism it is significantly decreased. The metabolic rate to accelerate the formation of nuclear cells and secretion of Cystatin C may be the direct cause of higher Cystatin C levels in Hyperthyroid patients.

Our findings have indicated the magnitude of impact of thyroid hormones on Cystatin C. This is in agreement with the study by Wiesli et al.(2003).They demonstrated that, Cystatin C levels reduced in subclinical hypothyroid patients and changes in Cystatin C levels are reversible, they further observed Cystatin C values came to normal level in the same patients after treating the cases to attain euthyroid state. Others have demonstrated the same effect in overt hypothyroidism also. Kotajima N et al. (2010) have tried to elucidate the mechanisms involved in the regulation of Cystatin C concentration in thyroid dysfunction by studying the effects of 3,5,3'-tri-iodothyronine (T3) and TGF- β 1 on Cystatin C production in cultured human hepatoblastoma (Hep G2) cells. These cells are known to express nuclear T3 receptor & TGF- β 1 receptors. And their results appear to be the first to note that, T3 stimulated the production of Cystatin C in Hep G2 cells in a dose dependent manner in vitro.

Ozden TA et al. (2008) also found significantly lower serum cystatin C levels in hypothyroid group. They suggested that thyroid hormones have significant effects on renal hemodynamics, renal handling of salt & water and on the active tubular transport of Na^+ , K^+ and H^+ . It is possible that tubular creatinine secretion is diminished in hypothyroidism, thereby increasing the serum creatinine concentrations. They reported a slight decrease in GFR in patients with hypothyroidism, which improved significantly after treatment. Thyroid hormones may influence Cystatin C levels by altering their production rate. Since Cystatin C is produced at a constant rate that is independent of age, gender, muscle mass and external factors like inflammation, they may serve as markers of thyroid function. Also, serum CysC levels were found to be significantly correlated with ft4 in hypothyroid and euthyroid status in their study.

These findings indicate that serum Cystatin C levels may be a marker of thyroid function. The low levels of Cystatin C in subclinical hypothyroidism and the increase in serum Cystatin C levels after L-T4 therapy in overt patients as well as the significant correlation between ft4 and Cystatin C levels has been also shown in some other studies.

V. CONCLUSION

From the present study it can be concluded that in Subclinical Hypothyroidism the activity of Cystatin C is decreased. Similar observations are related with Atherogenesis i.e. decreased serum levels of cystatin C are found to be associated with atherosclerotic lesions.

Thus in conclusion it can be stated that the severity of hypothyroidism together with hypercholesterolemia, may explain and contribute to atherosclerosis. This might have

diagnostic significance for the prediction of cardiovascular diseases. Therefore, we recommend screening of serum cystatin C levels as valuable markers in subclinical hypothyroid subjects to predict the possibility of development of any cardiovascular complications accompanying Hypothyroidism.

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