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Original Article

Biochemistry Section

Non High Density Lipoprotein Cholesterol, a Simple and Reliable Marker to Assess Cardiovascular Disease Risk in Hypothyroid Patients

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ABSTRACT

Introduction: Thyroid hormones regulate growth, metabolism and cellular differentiation in our body. Thyroid disorders are among the commonly encountered endocrinopathies across the globe in the recent past. Hypothyroidism is prevalent to the extent of 9.45-11.73% among urban inland regions of India as reported in a recent survey. Thyroid hormones influence lipid metabolism. The dyslipidemia in hypothyroidism presents as high serum Total Cholesterol (TC) levels, elevated Low Density Lipoprotein-Cholesterol (LDL-C) levels, hypertriglyceridemia and Increased Triglyceride (TGL)- Rich Lipoproteins or Remnant Lipoproteins (RLPs). Non High Density Lipoprotein-Cholesterol (Non-HDL-C) reflects the cholesterol content of these RLPs. The potential of Non-HDL-C seems to be underutilised in patients with hypothyroidism.

Aim: To know the levels of Non-HDL-C in hypothyroid patients as compared to normal subjects and to assess its reliability to predict the future cardiovascular risk in hypothyroid patients.

Materials and Methods: The study was a case-control study, that was conducted in District Hospital, Chamarajanagar, Karnataka, India, between December 2019 to September 2020, involving two groups. The first group were the cases, consisting of 50 hypothyroid patients between 18 and 55 years of age, 50 healthy age and sex matched controls were selected and inducted into this study. The 'Case group was further divided into "Known cases" and 'New cases', based on the disease course. The 'New cases' are further classified into group of 'Overt Hypothyroids' and 'Subclinical hypothyroids'. In all the subjects of present study, serum TC and serum HDL-Cholesterol (HDL-C) was estimated. The value of HDL-C was subtracted from TC level and Non-HDL-C level was calculated for all the study subjects. The values of the lipid fractions in both groups and in the subgroup were tabulated and compared using suitable statistical tool.

Results: In the present study it was noted that Non-HDL-C was significantly high in hypothyroid patients when compared to healthy controls (p=0.02). Also, the newly diagnosed hypothyroid patients had significantly high Non-HDL-C levels than controls (p=0.01). The subgroup of 'Known cases' who were on Levo-thyroxine replacement did not show significantly elevated Non-HDL-C levels. Overt Hypothyroid (OH) patients had higher values of Non-HDL-C than those with Subclinical Hypothyroidism (SCH).

Conclusion: At each level of the present study, (controls vs cases, known vs new cases and OH vs SCH) non-HDL-C has emerged in an expected pattern, which is consistent with pathophysiology, course and severity of the disease. Therefore, present study suggest the feasibilty of non-HDL-C replacing the traditional lipid profile assays for assessment of dyslipidemia in hypothyroid patients.

Keywords: Atherosclerosis, Dyslipidemia, Overt hypothyroidism, Sub clinical hypothyroidism

INTRODUCTION

Thyroid hormones play a pivotal role in governing the major body functions like growth, development and metabolism. Triiodothyronine (T3) and thyroxine (T4) are the two main hormones secreted by thyroid gland. Thyroid Stimulating Hormone (TSH), secreted by the anterior pituitary determines the production and release of thyroid hormones into the circulation. Thyroid disorders are among the commonly encountered endocrinopathies across the globe in the past few decades. Iodine is required for the synthesis of thyroid hormones and iodine discrepancy in the diet has major impact on the prevalence of thyroid dysfunction among the population of a given region. Hyperthyroidism and hypothyroidism are associated with iodine replete and deplete states respectively. The global prevalence of OH ranges between 0.2-1.3% as per latest data available. OH prevalence ranges between 0.2-5.3% in Europe and 0.3-3.7% in the USA depending on the definition used and population studied [1]. Hypothyroidism is more common in females than males. A large study involving 33 lakh adults across India has revealed that 32% of the study group was affected by some form of thyroid disorder, which is alarming. Despite the salt iodisation program, a Government of India initiative, hypothyroidism is prevalent to the extent of 9.45-11.73% among urban inland regions as reported in a recent survey [2].

Hypothyroidism, a condition with features of slow metabolic rate is diagnosed by laboratory assessment of thyroid hormones. OH presents with an elevated TSH and T3, T4 levels below the reference ranges [3]. SCH refers to biochemical evidence of thyroid hormone deficiency in patients who have few or no apparent clinical features of hypothyroidism. SCH is defined biochemically by TSH concentration above the upper limit of the reference range, with thyroid hormone levels that remain within the reference range [4]. Iodine deficiency remains a common cause of hypothyroidism worldwide. In areas of iodine sufficiency, autoimmune disease and iatrogenic causes are foremost aetiologies causing hypothyroidism [3].

Thyroid hormones influence lipid metabolism. T3 is known to increase both cholesterol synthesis and uptake by the cells. The net effect of the same results in a balance between synthesis and clearance of cholesterol thereby maintaining normal serum cholesterol levels

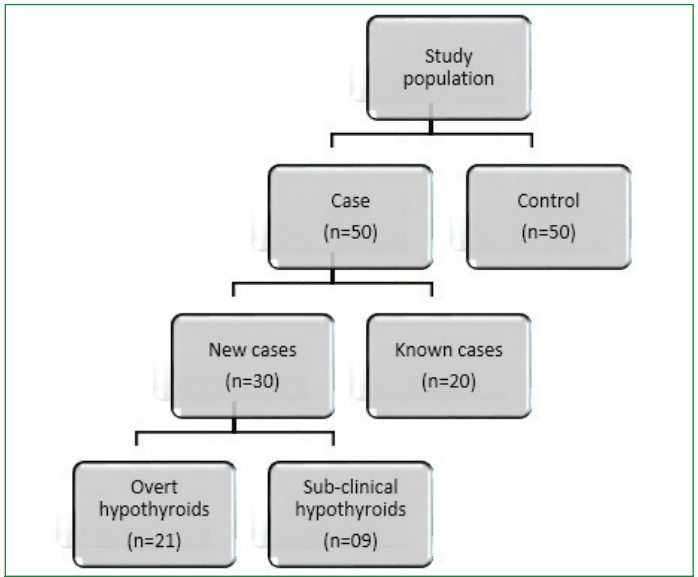
in euthyroid state [5]. OH is a secondary cause of dyslipidaemia [6]. Alterations in lipoprotein fractions have also been reported in patients of SCH [7]. The dyslipidemia in hypothyroidism presents with high serum TC levels, elevated LDL-C levels, hypertriglyceridemia, and increased triglyceride rich lipoproteins or RLPs [8]. Non-HDL-C reflects the cholesterol content of these RLPs [9].

Non-HDL-C quantifies apolipoprotein-B containing lipoproteins which include LDL, Very Low-Density Lipoprotein (VLDL), Intermediate-Density Lipoprotein (IDL), chylomicrons (CM), and their TG-rich lipoprotein remnants. This qualifies Non-HDL cholesterol as a surrogate marker of all atherogenic Apolipoprotein B [10]. It can simply be calculated by subtracting HDL-C level from TC level. The estimations of these two lipid parameters can be done on a non fasting sample which provides convenience for the patients. The potential of Non-HDL cholesterol seems to be underutilised in patients with hypothyroidism. There are not many researches that reports Non-HDL cholesterol levels in hypothyroid patients. This study purports to know the levels of Non-HDL cholesterol in Hypothyroid patients as compared to normal patients and also to assess whether Non-HDL-C serves a reliable Cardiovascular Disease (CVD) risk marker in hypothyroid patients.

MATERIALS AND METHODS

The present study was a case-control study which was conducted in District Hospital, Chamarajanagar, Karnataka, India, between December 2019 to September 2020. The Institutional Ethics Committee approval was obtained prior to commencement of the study (Ethical Clearance Certificate no. CIMS/IEC-02/2018-2019 dated 06/09/2019). An informed consent was taken from all the subjects recruited into the study.

The cases of the study comprised of patients who attended General Medicine outpatient department. The case Group consisted of 50 hypothyroid patients between 18 and 55 years, for which corresponding age and gender matched 50 healthy 'Controls" were selected. The control group were those individuals with normal reference range of TSH 0.4-4.2 µIU/mL, T3 70-204 ng/dL and T4 of 5.5-11 µg/dL. [Table/Fig-1] represents the study group and their different sub divisions.



[Table/Fig-1]: Study groups and their sub classifications.

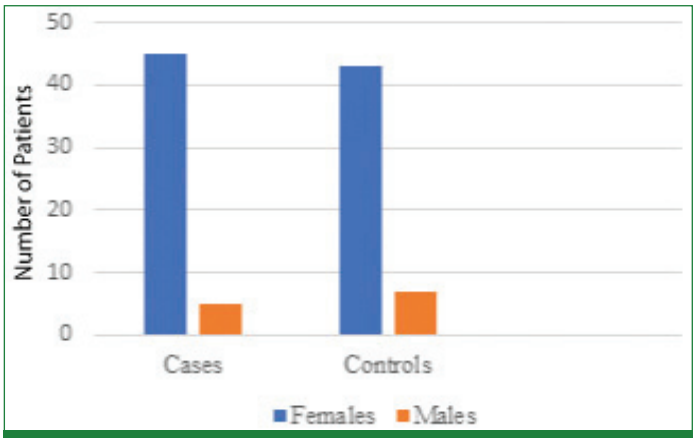
The case group was further divided into two groups as shown in [Table/Fig-1]. Nine patients were SCH, which is defined by their TSH levels which was found to be between 4.2 and 10 µIU/mL and T3, T4 values within the normal reference ranges [11]. The remaining 21 of this subgroup consisted of OH patients. Hypothyroid patients who were pregnant or had history of any medical or surgical illness were excluded from the study. Thyroid profile tests were processed on Chemiluminescence immunoassay based autoanalyser Maglumi-1000. The venous blood sample of both hypothyroid patients and healthy controls were drawn under all aseptic precautions. This sample was utilised to estimate serum TC and HDL-C levels. Both the investigations were analysed on photometry based fully automated general chemistry analyser from Transasia company, ERBA XL-640. Serum TC was estimated by cholesterol oxidase method, serum HDL-C was measured by direct method. After estimation, the value of HDL-C was subtracted from TC level and Non-HDL-C level was calculated for all the study subjects. The normal reference range for HDL-C was taken as ≤130 mg/dL [12]. The values of the lipid fractions in both groups were tabulated and compared using suitable statistical tool.

STATISTICAL ANALYSIS

The mean value and Standard Deviation (SD) for all the biochemical parameters was calculated. The Mean±SD between the cases and controls were compared using student's t-test. The correlation between biochemical parameters were performed using Pearson's correlation analysis. All statistical analysis was done at 5% level of significance.

RESULTS

The Mean±SD of age of the hypothyroid patients in cases and of healthy adults in control group were 35.46±9.32 and 33.06±8.86, respectively. [Table/Fig-2] depicts the gender distribution of study population in both the groups.



[Table/Fig-2]: Gender distribution of study population.

The Mean±SD of biochemical parameters studied were compiled and compared between controls and cases as shown in [Table/Fig-3].

[Table/Fig-4,5] highlights the lipid parameters values of control vs known cases and control vs new cases respectively.

Biochemical parameter	Control group (n=50)	Case group (n=50)	p-value
Serum total T3 (ng/dL)	160.54±30.50	151.01±39.07	0.17
Serum total T4 (µg/dL)	10.55±12.38	6.09±2.14	0.01*
Serum TSH (µIU/mL)	2.36±0.98	29.63±30.59	<0.0001**
Serum total cholesterol (mg/dL)	168.16±34.87	185.48±43.3	0.03*
Serum HDL cholesterol (mg/dL)	53.12±12.05	52.14±11.35	0.67
Non-HDL cholesterol (mg/dL)	114.7±34.78	133.13±42.57	0.02*

[Table/Fig-3]: Biochemical investigations of the two major study groups.

Biochemical parameter	Control group (n=50)	Known cases (n=20)	p-value
Serum Total cholesterol (mg/dL)	168.16±34.87	177.4±28.29	0.29
Serum HDL cholesterol (mg/dL)	53.12±12.05	53.51±12.65	0.90
Non- HDL cholesterol (mg/dL)	114.7±34.78	122.86±23.06	0.33

[Table/Fig-4]: Comparison of lipid parameters between control group and known case subgroup of cases.

Biochemical parameter	Control group (n=50)	New cases (n=30)	p-value
Serum total cholesterol (mg/dL)	168.16±34.87	190.86±50.72	0.02*
Serum HDL cholesterol (mg/dL)	53.12±12.05	51.23±10.52	0.47
Non-HDL cholesterol (mg/dL)	114.7±34.78	139.3±51.14	0.01*

[Table/Fig-5]: Comparison of lipid parameters between control group and newly diagnosed cases subgroup of cases.

[Table/Fig-6] gives insight about lipid particle level variation between two sub-subgroups of "New cases" subgroup.

[Table/Fig-7] shows correlation analysis between TSH and Non-HDL-C levels in the two major groups of present study.

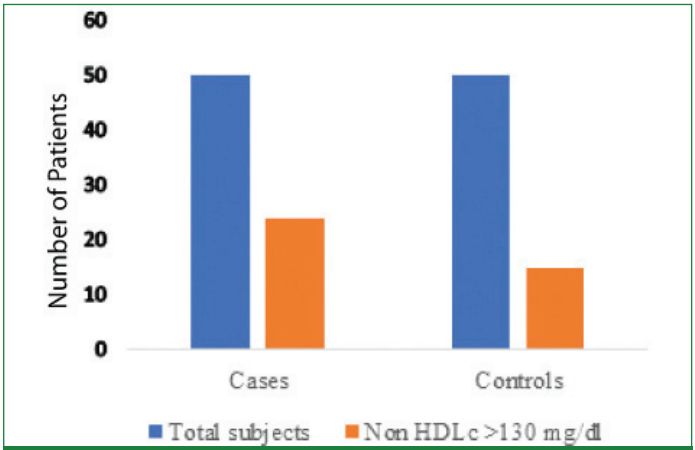
When the number of patients with Non-HDL-Cholesterol >130 mg/dL was analysed between the two study population the following observation emerged as depicted in [Table/Fig-8].

Biochemical parameter	Overt hypothyroid (n=21)	Subclinical hypothyroid (n=09)	p-value
Serum total cholesterol (mg/dL)	197.42±58.10	175.55.±22.58	0.28
Serum HDL cholesterol (mg/dL)	50.36±9.70	53.25±12.63	0.50
Non- HDL cholesterol (mg/dL)	146.58±57.90	122.3±25.18	0.23

[Table/Fig-6]: Comparison of lipid parameters between overt hypothyroid and subclinical hypothyroid patients of the newly diagnosed cases subgroup of cases.

Pair	Pearson's correlation	p-value
Serum TSH v/s Non-HDL cholesterol in controls	0.21	0.13
Serum TSH v/s Non-HDL cholesterol in cases	0.14	0.32

[Table/Fig-7]: Pearson's correlation between TSH and Non-HDL cholesterol in both cases and controls.



[Table/Fig-8]: Number of subjects with Non HDLC >130 mg/dL in cases and controls.

DISCUSSION

Thyroid hormones have major influence on synthesis and metabolism of lipids. They are also responsible for mobilisation of lipids. Thus, decrease in thyroid hormones level impact lipid harmony in the body. The derangement in circulating lipid particles in OH is now a well-established fact. There is elevation in serum TC, LDL-C, TGL, Apolipoprotein B and lipoprotein (a) levels in OH [13]. Hypertriglyceridemia, hypercholesterolemia are seen in association with SCH [14]. The abnormal lipid fractions predispose to atherogenesis and lead to CVD which can be fatal. Therefore screening hypothyroid patients periodically for CVD risk markers becomes essential. Currently lipid profile test which include estimation of different fractions like serum TC, LDL-C, HDL-C and TGL are routinely done to assess CVD risk in individuals. Certain studies have recommended Non-HDL-C for evaluation of CVD risk in diabetics [15]. It is superior to the conventional lipid profile test in many ways. The utility of this parameter in detecting and monitoring atherogenesis risk in hypothyroid patients' needs to be established, which was primary intention of present study.

The thyroid profile test was the basis on which two groups of present study were stratified, as an obvious outcome of this, significant differences in T4 and TSH levels was observed between the two groups. When mean and SD values of TC, HDLC and Non-HDLC was compared between case and control group, it was observed that the serum TC levels were significantly high among hypothyroid patients (p=0.03). Non-HDLC, the parameter of interest, was found to be significantly increased in hypothyroid patients than in healthy controls (p=0.02). This is in accordance with observations made by Kalaiperumal R et al., in a similar study [16]. Further when three lipid fractions of the control group with sub groups of cases were compared, individually, it was found that the serum TC and Non-HDLC levels were significantly higher in newly diagnosed cases (p=0.02 and p=0.01, respectively). Though the levels of serum TC and Non-HDLC was higher in known cases subgroup than in controls, no increase to the extent of being statistically significant was observed. This can be explained by the finding made by Ito M et al., where in they observed that Levo-thyroxine replacement may reduce the Non-HDLC level concentrations in patients with hypothyroidism [17]. All the hypothyroid patients in the 'known cases' sub-group of cases were already on thyroxine replacement therapy. The 'new cases' were further subdivided into OH and SCH group it was observed that the mean±SD on TC was higher in OH patients (197.42±58.10) than in SCH patients (175.55±22.58). The Non-HDLC in OH patients was 146.58±57.90 as against 122.3±25.18 in SCH group. It is clear that the lipid fractions are rising quantitatively, though not to the extent of being statistically significant. There have been studies which have reported that the lipid status worsens with failing thyroid function. The lipid alterations become increasingly significant as SCH progresses to OH [18]. In present study no kind of association between thyroid hormones and lipid parameters were observed. The overall percentage of subjects in control group with levels of Non-HDL-C >130 mg/dL are 30% compared to 48% among case group.

Thyroid hormones depletion affects lipid metabolism in our body. The abnormal increase in TC and LDL-C levels in hypothyroidism is a result of impaired gene modulation and cell signaling pathways mediated by thyroid hormones due to which abnormal expression of LDL receptor and 3-hydroxy 3-methyl glutaryl Co-A reductase genes occurs. There is an imbalance that develops between clearance and synthesis of cholesterol, leading to increased circulating fractions of serum TC and LDL-C [19].

There is an increase in plasma TGL and TGL rich lipoprotein levels in hypothyroidism. This occurs because thyroid hormones are required for maintaining optimal activity of Cholesterol Ester Transfer Protein (CETP), Hepatic Lipase (HL) and Lipoprotein Lipase (LPL) enzymes. The decreased activity of these enzymes leads to slow clearance of TGL from circulation and accumulation of RLP [20]. The RLPs are identified as atherogenic and indicates the possibility of a future cardiovascular assault.

This assault can be averted with a timely detection of dyslipidemia by a simple agent which represents the atherogenic lipids. Non-HDL-C can serve as one such tool. It is superior to LDL-C in predicting major adverse cardiovascular events [21]. It is more compliant test, as fasting sample is not required for the assay and is cost-effective since it involves only two lipid parameters that needs to be analysed [21]. The latter two factors are hugely motivating to patients to get the screening done especially in a place like Chamarajanagar where more than 50% of the population visiting the District Hospital is from below poverty line strata.

Limitation(s)

The limitation of the present study was its small samples size, because of which the results cannot be applied to the larger

population. Authors recommend similar study to be undertaken in a larger group. To prove the superiority of Non-HDL-C over LDL-C, authors have not estimated LDL-C levels among the subjects which is another drawback.

CONCLUSION(S)

At each level of the present study, (controls vs cases, known vs new cases and OH vs SCH) Non-HDL-C has emerged in an expected pattern which is consistent with pathophysiology, course and severity of the disease. Therefore, present study report suggests the feasibility of Non-HDL-C replacing the traditional lipid profile assays for assessment of dyslipidemia in hypothyroid patients.

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