ORIGINAL ARTICLE

Evaluation of LDL-Cholesterol / HDL-Cholesterol Ratio as Predictor of Dyslipidemia in Subclinical Hypothyroidism

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Abstract:

Background: Subclinical hypothyroidism is defined as a serum TSH concentration above the upper limit of the reference range when serum T3 and T4 concentrations are within reference ranges. Subclinical thyroid disease is a laboratory diagnosis. Patients with subclinical disease have few or no definitive clinical signs or symptoms of thyroid dysfunction. It has been associated with higher levels of some cardiovascular risk factors. Despite some conflicting results, many studies have found that subjects with subclinical hypothyroidism have total cholesterol and low density lipoprotein cholesterol levels higher than euthyroid subjects. The association between subclinical hypothyroidism and dyslipidemia is well known. *Aims and Objectives*: This study is an attempt to find the importance of Low Density Lipoprotein – Cholesterol / Higher Density Lipoprotein - Cholesterol (LDL-C/HDL-C) ratio rather than measurement of individual lipid profile parameters in bringing to light the dyslipidemic state associated with subclinical hypothyroidism. *Materials and Methods*: We studied 30 subclinical hypothyroid cases with age above 35 yrs and 30 age matched euthyroid controls. Serum T3, T4, TSH were estimated by ELISA method, serum total cholesterol, HDL Cholesterol by enzymatic CHOD-PAP method, and LDL cholesterol using Friedewald formula. *Results*: We found the significant increase in the serum levels of TSH ($p < 0.001$), Total cholesterol ($p<0.001$), LDL cholesterol ($p<0.001$), and LDL-C/HDL-C ($p<0.001$), Systolic blood pressure and diastolic blood pressure ($p<0.001$). There was no significant change in the levels of serum T3, T4, HDL- cholesterol. *Conclusion*: Increased levels of total cholesterol, LDL cholesterol and increased LDL-C/HDL-C ratio are seen in patients with subclinical hypothyroidism. LDL-C/HDL-C ratio is a better indicator for dyslipidemia in subclinical hypothyroid cases.

Keywords: Cardiovascular risk, Dyslipidemia, Subclinical hypothyroidism (SCH), LDL-C/HDL-C ratio.

Introduction:

Subclinical hypothyroidism (SCH) is defined as a serum TSH concentration above the statistically defined upper limit of the reference range when serum T4 (T4) concentration is within its reference range [1]. A 2-fold change in free thyroxine will lead to a 100-fold change in TSH. Even slight decrease in T4 levels within the normal range will lead to increase in serum TSH above the normal range. So, measurement of serum TSH is the important test for diagnosis of mild thyroid failure when the peripheral thyroid hormone levels are within normal laboratory range [2]. Subclinical hypothyroidism is a laboratory diagnosis. Patients with subclinical thyroid disease have few or no clinical signs or symptoms of thyroid dysfunction [1].

Subclinical hypothyroidism is a common problem, with a prevalence of 3% to 8% in the population without known thyroid disease [3]. The clinical significance and the treatment for mild elevation of serum TSH (<10mIU/L) [1] and the exact upper limit of normal range for the serum TSH level remain subjects of debate [4]. Levothyroxine therapy is generally preferred to be appropriate for TSH levels above 10mIU/L [5]. But, starting the therapy for patients having a serum TSH level lower than 10mIU/L is controversial. Some authors argue for routine management and some for selective therapy [6].

Subclinical hypothyroidism is associated with higher levels of some cardiovascular risk factors. Though the results are conflicting, many studies have found that subjects with subclinical hypothyroidism have higher total cholesterol and low density lipoprotein cholesterol levels as compared to euthyroid subjects [7].

Mechanisms underlying the development of hypercholesterolemia in hypothyroidism include decreased fractional clearance of LDL-C by a reduced number of LDL-C receptors in the liver in addition to decreased receptor activity. The catabolism of cholesterol into bile is mediated by the enzyme cholesterol 7 –hydroxylase. This liver specific enzyme is negatively regulated by T3 and may contribute to the decreased catabolism and increased levels of serum cholesterol associated with hypothyroidism. The increased serum lipid levels in subclinical hypothyroidism as well as in overt disease are potentially associated with increased cardiovascular risk. The thyroid hormone replacement therapy is found to restore euthyroidism and reverses the risk ratio [8].

In hypothyroidism, endothelial dysfunction and impaired vascular smooth muscle relaxation lead to increased superior venacaval resistance. These effects lead to diastolic hypertension in nearly 30% of patients, and thyroid hormone replacement therapy restores endothelial-derived vasorelaxation and blood pressure to normal in most [8].

Subclinical hypothyroidism has been associated with increased risk for atherosclerosis. The data in subjects with subclinical hypothyroidism with coronary heart disease (CHD) are conflicting [7]. Although established risk factors explain most cardiac events, significant attention has been focused on alternative biochemical markers to aid in identifying those at risk of clinical cardiac events [9]. This study underlines the importance of LDL-C/HDL-C ratio rather than measurement of individual lipid profile parameters in bringing to light the dyslipidemic state associated with SCH.

Material and Methods:

The study is carried out in the Department of Biochemistry, BLDEU'S Shri. B. M. Patil Medical College Hospital and Research Centre, Bijapur (Karnataka) India. We studied 30 subclinical hypothyroid cases aged above 35 years and 30 euthyroid controls from the general population according to the inclusion and exclusion criteria mentioned below. This study was approved by the Institutional Ethics Committee. All the subjects gave an informed consent before undergoing further investigations.

Inclusion criteria: Subclinical hypothyroidism cases having TSH in the range of 4.50 to 14.99 mlU/L, T3 and T4 within normal limits. The euthyroid controls having normal TSH [0.3-4.5 mlU/L.]

Exclusion criteria: Known hypothyroidism cases, thyroidectomy cases, patient with external radiation, previous radioactive iodine therapy, consumption of drugs known to cause SCH, primary or secondary dyslipidemia, patients with diabetes mellitus**,** patients with other systemic illness, renal and hepatic failure cases**,** patients on statins were excluded from the study. The subjects were selected from the cases referred to our clinical biochemistry laboratory by other departments (Medicine, Surgery, Gynecology, psychiatry) requesting for thyroid profile, suspecting different thyroid disorders. We performed thyroid profile on such cases and included only those subjects that came under the category of subclinical hypothyroidism. As subclinical hypothyroidism is purely a lab diagnosis we further requested these patients for lipid profile explaining to them the aim of our study.

Venous blood samples were drawn at 8 a.m. following a 12 hours fast, in a plain bulb from the subjects, with all the aseptic precautions. Blood samples were centrifuged within 30 minutes at 3000 rpm for 5 min. and serum was separated. Serum samples were stored at -20°C until assayed serum T3, T4, TSH were estimated by ELISA method [10-12]. The principle of serum TSH estimation by ELISA method - Specific anti-TSH antibodies are coated on to micro titration wells. Test sera are applied. Then goat anti-TSH labelled with Horseradish peroxidase enzyme (conjugate) is added. If human TSH is present in the sample it will combine with the antibody on the well and the enzyme conjugate, resulting in the TSH molecule being sandwiched between the solid phase and the en-

zyme linked antibodies. After incubation the wells are washed to remove the unbound labelled antibodies. On addition of the substrate, a color will develop only in those wells in which the enzyme conjugate is present, indicating the presence of TSH. The enzyme reaction is stopped by the addition of dilute hydrochloric acid and the absorbance is measured at 450 nm

Principle of serum T3 estimation by ELISA method: Goat anti-mouse IgG antibody is coated on to micro titration wells. Test sera are applied along with antibody reagent. T3 enzyme conjugate is added which competes with the serum T3 for available binding sites on the solid phase. After incubation, the wells are washed to remove any unbound T3 or T3 enzyme conjugate. On addition of the substrate, a color develops only in those wells in which enzyme is present indicating a lack of serum T3. The reaction is stopped and the absorbance is then measured at 450nm.

Principle of serum T4 estimation by ELISA method: Specific anti-T4 antibodies are coated on to microtitration wells. Test sera are applied. T4 with Horseradish peroxidase enzyme (conjugate) is added which competes with the serum T4 for available binding sites on the solid phase. After incubation, the wells are washed to remove any unbound T4 or T4 enzyme conjugate. On addition of the substrate, a colour develops only in those wells in which enzyme is present indicating a lack of serum T4. The reaction is stopped by the addition of dilute hydrochloric acid and the absorbance is then measured at 450nm.

Principle of serum total cholesterol estimation by enzymatic CHOD-PAP method: Cholesterol esterase hydrolyzes cholesterol esters to free cholesterol and fatty acids. Cholesterol is oxidized by cholesterol oxidase forming hydrogen peroxide and cholest-4ene-3one. In presence of peroxidase, hydrogen peroxide is formed which brings about oxidative coupling of phenol and antipyrine to form red coloured quinoneimine dye. Intensity of colour generated is directly proportional to total cholesterol concentration.

Principle of serum HDL-C estimation by enzymatic CHOD-PAP method: Chylomicrons, VLDL and LDL

Table 1- Comparison of Parameters between Subclinical Hypothyroid Cases and Euthyroid Controls

Parameter	Controls (N=30)	SCH Patients (N=30)
T3 (nmol/l)	001.85 ± 00.96	001.52 ± 00.38*
T4 (nmol/l)	089.41 ± 22.04	082.72 ± 17.93
TSH (mlU/L)	002.58 ± 01.06	008.11 ± 02.54**
TC (mg/dl)	179.06 ± 37.10	268.10 ± 32.64**
LDL (mg/dl)	127.50 ± 35.63	189.89 ± 42.93**
HDL (mg/dl)	046.53 ± 19.89	041.17 ± 08.35
LDL/HDL	003.1 ± 00.70	005.08 ± 02.74**
SBP (mm Hg)	122.20 ± 04.01	126.87 ± 05.72**
DBP (mm Hg)	081.13 ± 04.4	088.40 ± 04.14**

*** Indicates p<0.001- Highly Significant, * p<0.01- Significant. T3=Tri-iodothyronine, T4=Tetra iodothyronine, TSH=Thyroid stimulating hormone, TC=Total cholesterol, LDL=Low density lipoproteins, HDL=High density lipoproteins, SBP= Systolic Blood Pressure, DBP= Diastolic Blood Pressure.*

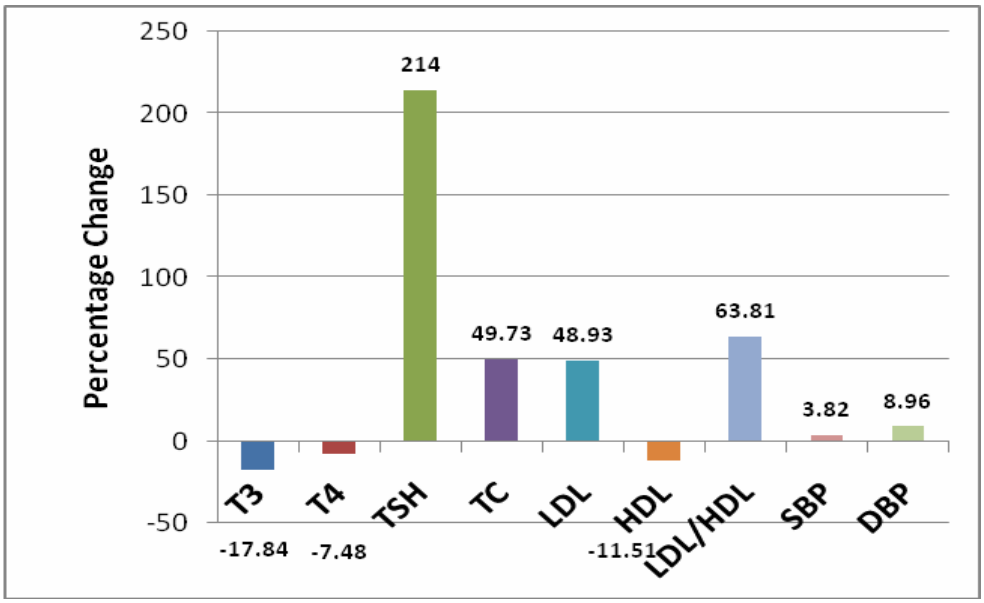

Fig. 1- Shows the Percentage Change of the Biochemical Parameters in Study Group as Compared to Controls

are precipitated by adding phosphotungstic acid and magnesium ions to the sample. Centrifugation leaves only HDL in the supernatant. The cholesterol content in it is determined enzymatically on Mispa semi autoanalyser [13].

LDL-C was calculated by using Friedewald formula (LDL=TC-HDL-TG/5) [14].

The data is presented as mean ± SD, statistical analysis was carried out using unpaired students 't' test for all variables. P value of 0.05 or less was considered as statistically significant.

Results:

Table 1 shows that serum mean levels of TSH, TC, LDL-C, LDL-C/HDL-C, SBP, DBP were significantly increased in SCH patients as compared to controls where as T3 , T4, HDL-C did not show statistically significant difference as compared to controls.

Discussion:

Subclinical hypothyroidism is more common than overt hypothyroidism. In recent times it is being diagnosed more frequently than overt hypothyroidism [15]. Despite of this disorder being more common, its clinical significance is still debatable. Still there is controversy pertaining to routine screening of SCH so as to prevent it from progressing to overt hypothyroidism [16]. The continuing debatable aspects regarding subclinical hypothyroidism are, the associated dyslipidemic state, cardiovascular risk, neuromuscular and psychiatric dysfunction, underlying proinflammatory state, etc. The clear concepts in this aspect are yet to be obtained. [17, 18].

The relationship between SCH and serum lipids remains controversial. Several studies, have reported variable and inconsistent increase in total cholesterol, LDL-C, higher and inconsistent changes in serum HDL-C [19-21]. SCH was not associated with hyperlipidemia, in Whickham survey [22], where as NHANES III reported, higher levels of mean cholesterol in SCH subjects as compared to euthyroid subjects but no difference was reported in LDL-C or HDL-C [23]. Marwaha et al studied dyslipidemia in subclinical hypothyroidism in Indian population, and reported increase in total cholesterol and LDL cholesterol in subclinical hypothyroidism with TSH >

10mlU/L where as no significant difference was seen in lipid profile parameters when TSH levels were less than 10 mlU/L as compared to controls [24]. In the Rotterdam study, total cholesterol was lower in SCH women as compared to euthyroid women [25]. Bindels et al [26] estimated that an increase of 1 mlU/L in serum TSH was associated with a rise in serum cholesterol of 0.09mmol/L (3.5 mg/dl) in women and 0.16mmol/L (6.2 mg/dl) in men.

The occurrence of cardiovascular event is not solely dependent on atherogenic lipoproteins, but is based on the balance between atherogenic and athero-protective lipoproteins [27]. The existing focus on LDL-C as the primary cause in atherogenesis is now diverted by the more efficient ratio of the lipid parameters LDL-C/HDL-C ratio [28]. 1-unit increase in the LDL-C/HDL-C ratio is associated with a 75% increase in risk of MI [29]. Many studies have found that the LDL-C/HDL-C ratio is a more efficient tool to monitor the effect of lipid lowering therapies. The LDL-C/HDL-C is a better predictor of dyslipidemia in assessing the risk of heart disease than LDL-C alone. The ratio of LDL-C/HDL-C reflects the movement of cholesterol by entry and depart in the arterial intima [30]. Several studies have also shown that the LDL-C/HDL-C ratio is not affected by dietary cholesterol [31, 32].

We observed that the percentage change for LDL-C/HDL-C (68.31%) which was more as compared to individual parameters like TC(49.73%), LDL-C (48.93%) (Fig. 1). Our study is similar to the study done by Mala Mahto et al [9] which has shown that LDL/HDL ratio is more significant between the two groups, but individual parameters i.e. TC, HDL-C, LDL-C are not significant. But our study has shown statistically significant values for TC and LDL-C as well. Our study has revealed a significant p value of < 0.001 when the LDL-C/HDL-C ratio is compared between the two group and also TC and LDL-C are significant. Our study is similar to the study done by B. U. Althaus, J. J. Staub et al [33] which has shown a significant LDL-C/HDL-C ratio but they have observed no significant value for HDL-C and LDL-C but our study has shown significance for TC and LDL-C as well.

Fernandez M and Webb D have shown that the ratio between these particles predicts cardiovascular disease (CVD) risk better than the isolated lipoprotein sub fractions [34]. Our study is in accordance with the study done by Fernandez M et al and Tian et al [30]. An increase in LDL/HDL ratio points for initiating lipid-lowering therapy. The current NCEP guidelines recommend levels of LDL-C and HDL-C that represent a ratio of about 2.5. Current research suggests risk of death from cardiovascular disease begins to increase significantly around a ratio of 3.3–3.7 [34]. This highlights the importance of measurement of all the lipid fractions individually and calculating the ratio of the atherogenic and atheroprotective fractions. This would reflect the actual balance between the two fractions & help in better prediction of a cardiovascular risk.

Our study has shown a significant difference for systolic and diastolic blood pressure between subclinical hypothyroid cases and euthyroid controls. These results are in contrast with the study done by A. Elisabeth Hak et al [25] which has shown no difference for blood pressure between the two groups. But the study done by Rafael Luboshitzky et al has not shown any significant difference for systolic blood pressure but has shown a significant difference for diastolic blood pressure when compared between the two groups [35]. Imaizumi et al have studied only systolic blood pressure which has shown no significant difference between the two groups [36]. This clearly indicates that increase in blood pressure attributes to the increased risk for cardiovascular diseases in subclinical hypothyroidism.

The results of this study conclude that the increased levels of total cholesterol, LDL cholesterol and increased LDL-C/HDL-C ratio are seen in patients with subclinical hypothyroidism. LDL-C/HDL-C ratio is a better indicator of dyslipidemia in subclinical hy-

pothyroid cases. This ratio can predict the cardiovascular risk in subclinical hypothyroidism and may potentially be used as a screening aid to detect and treat those SCH cases with a greater cardiovascular risk. A detailed further study is required with large population size.

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