INTRODUCTION
Hypothyroidism is defined as reduced activity of the thyroid gland which results in either reduced T3 or T4 which in turn leads to increase in TSH levels. Subclinical hypothyroidism is defined as a high serum TSH concentration and normal serum total/ free thyroxine (T4), triiodothyronine (T3) concentrations associated with few or no symptoms/signs of hypothyroidism. Hypothyroidism is one of the most common causes of secondary dyslipidemia, both overt hypothyroidism and subclinical hypothyroidism. Strong association of hypothyroidism and atherosclerosis is well known. Thyroid function significantly affects lipoprotein metabolism, therefore cardiovascular (CVD) risk. A linear increase in total cholesterol, low-density lipoprotein cholesterol (LDL), triglycerides (TG) and a linear decrease in high-density lipoprotein cholesterol (HDL) levels have been observed with increasing TSH. Hypothyroidism is associated with premature atherosclerosis and increased prevalence of coronary diseases. Lipid abnormalities vary greatly between individuals and there is a relationship between subclinical hypothyroidism, dyslipidemia and cardiovascular diseases. Total cholesterol and LDL levels are increased in patients with hypothyroidism. Moreover, a decrease in Lipoprotein Lipase activity is found in hypothyroidism, decreasing the clearance of triglyceride rich lipoproteins. Hypothyroid patients may also exhibit elevated levels of HDL mainly due to increased concentration of HDL2 Particles and due to a reduction of Hepatic lipase activity and decreased in HDL2 catabolism. Effect of thyroid hormones on lipid profile: Thyroid hormones induce the 3-hydroxy-3- methylglutaryl coenzyme A (HMG-CoA) reductase, which is the first step in cholesterol biosynthesis. Moreover, triiodothyronine (T3) upregulates LDL receptors by controlling the LDL receptor gene activation which is done by the direct binding of T3 to specific thyroid hormone responsive elements (TREs). T3 also controls the sterol regulatory element-binding protein-2 (SREBP-2), which in turn regulates LDL receptor’s gene expression. T3 has also been associated with protecting LDL from oxidation. Thyroid hormones can influence HDL metabolism by increasing cholesteryl ester transfer protein (CETP) activity, which exchanges cholesteryl esters from HDL2 to the very low density lipoproteins (VLDL) and TGs to the opposite direction. In addition, thyroid hormones stimulate the lipoprotein lipase (LPL), which catalyzes the TG-rich lipoproteins and the hepatic lipase (HL), which hydrolyzes HDL2 to HDL3 and contributes to the conversion of intermediate-density lipoproteins to LDL and in turn LDL to small dense LDL (sdLDL). Another effect of T3 is...
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the up-regulation of apolipoprotein A-V, which plays a major role in TG regulation. Increased levels of ApoA-V have been associated with decreased levels of TGs. Proposed mechanisms for this effect include the decrease of hepatic VLDL-TG production and the increase of plasma LPL levels and activity, resulting in increase of lipoprotein remnant generation due to enhanced LPL-mediated lipolysis of VLDL-TG. There is also increased oxidation of plasma LDL and lipid peroxidation as a result of hyper-cholesterolemia and a decrease in the amount of T4 available to bind to LDL; this makes apolipoproteins vulnerable to the effects of free radicals through conformational changes. There are thus qualitative alterations in circulating lipoproteins that increase their atherogenicity. The above abnormalities of lipid metabolism associated with hypothyroidism predispose to the development of atherosclerotic coronary artery disease and cardiovascular diseases. Moreover, hypothyroidism increases plasma homocystein levels, which can be attributed to the hypothyroidism-induced decline of kidney function as well as impaired methylenetetrahydrofolate reductase activity. In addition, thyroid failure is strongly associated with arterial hypertension (especially diastolic) via sympatetic and adrenal activity, and increased vascular stiffness. Subjects with overt hypothyroidism also exhibit impaired endothelial function, increased uric acid and phosphate levels, all of which are associated with increased CVD risk. Initially the estimation of serum lipids like cholesterol, triglycerides, LDL and HDL were used to assess the risk of coronary artery disease. However, the inconsistency in the correlation between serum lipid profile and cardiovascular disease, led to the development of better indicators. Among them the estimation of serum apolipoproteins as a risk factor in coronary heart disease and other cardiovascular diseases and also as a marker has shown great promise.

Apo A-I and B are structural and functional protein components of lipoprotein particles that serve as transporters of cholesterol. Apo A-I and HDL are protective; Apo B and LDL are atherogenic. Increased Apo B/Apo A-I ratio was seen in CHD patients. The ratio reflects two powerful components of risk and provides a tool to express the balance between the proatherogenic and the antiatherogenic lipoproteins. More recent work however provides growing evidence that apo B and apo A-I are more effective indicators of cardiovascular risk.

Aim of this study was to estimate the levels of serum Apolipoprotein A-I, serum Apolipoprotein B and serum lipid profile and to find out Apo B/Apo A-I ratio and its role as cardiovascular risk indicator in patients with hypothyroidism.

MATERIALS AND METHODS

Study Design: The study was carried out at the Clinical Chemistry Laboratory of Biochemistry department at Medical College and S.S.G. Hospital, Baroda from July 2013 to November 2013 after obtaining Ethical clearance from the Institutional Ethics Committee for Human Research, Medical College and S.S.G. Hospital, Baroda. After taking consent, blood samples were collected in fluoride vacutainer for sugar estimation and in plain vacutainer for biochemical parameters. The subjects selected for the study were grouped as follows:

Inclusion criteria:

Group I – Control group (n=45) This group consisted of age and sex matched healthy subjects.

Group II – Patients with hypothyroidism (n=45) Newly diagnosed patients with hypothyroidism with age group of 30-65 years.

Exclusion criteria:
The following patients were excluded from the study:
1. Patients with coronary artery disease.
2. Patients on lipid lowering agents e.g. Statins.
3. Patients with history of stroke, intermittent claudication, peripheral vascular disease, carotid surgery, coronary artery bypasses graft surgery or PTCA.
4. Patient having H/O chronic alcohol consumption, hepatobiliary disorders or any other acute liver diseases and diabetes mellitus.

Thyroid function tests (S. TSH, total T3 and total T4) were performed by ELISA method. Estimation of serum Apo A-I and Apo B were done by Turbidimetric Immunoassay Method on fully automated biochemistry analyzer Miura-300. Serum lipid profile (Total cholesterol, triglyceride, HDL, LDL), liver function test and plasma glucose were measured on fully automated biochemistry analyzer. Serum VLDL and Apo B/Apo A-I ratio were calculated.

Statistical method: Data analysis was done by MedCalc version 11.5.0.0. Unpaired t-test was used to assess the significant difference in the means of the studied variables in the different groups. Interpretation was done according to p-value as follows:
- p < 0.05 is considered significant
- p ≥ 0.05 is considered not significant
- p < 0.0001 is considered highly significant.
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RESULTS
Table 1: Results of the control group and patients with hypothyroidism

<table>
<thead>
<tr>
<th></th>
<th>Group I (Controls)</th>
<th>Group II (Hypothyroidism)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>45</td>
<td>45</td>
<td>-</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>33/12</td>
<td>15/30</td>
<td>-</td>
</tr>
<tr>
<td>Average age (years)</td>
<td>43 ± 5</td>
<td>43 ± 6</td>
<td>-</td>
</tr>
<tr>
<td>S. TSH (mIU/L)</td>
<td>0.4-3.5</td>
<td>2.8 ± 1.1</td>
<td>16.3 ± 11.0</td>
</tr>
<tr>
<td>S. total T3 (mg/ml)</td>
<td>0.5-2.0</td>
<td>1.0 ± 0.7</td>
<td>0.5 ± 0.2</td>
</tr>
<tr>
<td>S. total T4 (mg/ml)</td>
<td>53-121</td>
<td>81 ± 30</td>
<td>50 ± 14</td>
</tr>
<tr>
<td>S. Total Cholesterol (mg/dl)</td>
<td>&lt; 200</td>
<td>166 ± 20</td>
<td>189 ± 30</td>
</tr>
<tr>
<td>S. HDL Cholesterol (mg/dl)</td>
<td>M:35-50</td>
<td>47 ± 6</td>
<td>43 ± 6</td>
</tr>
<tr>
<td>S. LDL Cholesterol (mg/dl)</td>
<td>&lt; 130</td>
<td>98 ± 16</td>
<td>119 ± 27</td>
</tr>
<tr>
<td>S. VLDL Cholesterol (mg/dl)</td>
<td>5-35</td>
<td>20 ± 8</td>
<td>27 ± 8</td>
</tr>
<tr>
<td>S. Triglyceride (mg/dl)</td>
<td>&lt;150</td>
<td>116 ± 51</td>
<td>140 ± 36</td>
</tr>
<tr>
<td>S. Apolipoprotein A-I (mg/dl)</td>
<td>M:107-177</td>
<td>130.7 ± 14.2</td>
<td>106.6 ± 17.2</td>
</tr>
<tr>
<td>S. Apolipoprotein B (mg/dl)</td>
<td>M:60-138</td>
<td>102.9 ± 16.4</td>
<td>124.8 ± 19.6</td>
</tr>
<tr>
<td>Apo B/Apo A-I ratio</td>
<td>&lt; 1.00</td>
<td>0.80 ± 0.17</td>
<td>1.21 ± 0.30</td>
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</tbody>
</table>

Table 2: Distribution of controls and cases according to Apo B/ Apo A-I ratio

<table>
<thead>
<tr>
<th>Apo B/ Apo A-I ratio</th>
<th>Controls (n=45)</th>
<th>Cases (n=45)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; 1.00 (high)</td>
<td>2 (4%)</td>
<td>32 (72%)</td>
<td></td>
</tr>
<tr>
<td>&lt; 1.00 (normal)</td>
<td>43 (96%)</td>
<td>13 (28%)</td>
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Percentage of patients with Apo B/Apo A-I ratio > 1.0 is significantly larger in cases when compared to controls (P < 0.05)

Figure 1: Correlation of S. TSH and S. Apo B/ Apo A-I ratio in cases (n=45, r=0.4426)

DISCUSSION
The study was done to assess serum apolipoprotein A-I and B levels, serum Apo B/ Apo A-I ratio, serum lipid profile and serum thyroid function test in patients with hypothyroidism. The study shows raised concentration of Apolipoprotein B and decreased concentration of Apo A-I in hypothyroid cases compared to the controls. These values also showed large inter-individual variations (SD for Apo A-I is 17.2, Apo B is 19.6) Although normal total cholesterol level in 60% patients and normal LDL level in 64% patients with hypothyroidism, serum Apo B/Apo A-I ratio is significantly high in 72% of hypothyroid cases and moderately correlates with S. TSH levels. The occurrence of hyperlipidemia contributes to the high incidence of coronary heart disease and increased cardiovascular mortality in patients with hypothyroidism. For over three decades it has been recognized that a high level of total cholesterol and low density lipoprotein cholesterol is a major risk factor for developing CHD but a considerable proportion of patients with CHD have normal levels of LDL and total cholesterol. Prospective and retrospective studies have suggested an independent association between high level of Apo B/ Apo A-I ratio (>1.00) and presence and extent of coronary artery disease and cardiovascular risk. More recently two studies, the AMORIS13 (17553 cases) study and the INTERHEART study14 (case control study of acute MI in 52 countries, 15152 cases and 14820 controls), have reported findings that the Apo B/Apo A-I ratio is a significantly better predictor of cardiovascular and stroke risk than any of the conventional cholesterol indices.

Hypothyroid patients are at risk of cardiovascular diseases because of altered lipid profile and altered apolipoprotein levels which lead to dyslipidemia and atherogenesis. Apolipoproteins A-I and B can be helpful in early prediction of the cardiovascular risk in hypothyroid patients. By treating such patients with hypolipidemic drugs and diet modification, the morbidity and mortality related to cardiovascular risk can be decreased.

CONCLUSION
The findings shows that serum Apo B/ Apo A-I ratio is high and could contribute towards increased the risk of cardiovascular diseases in patients with hypothyroidism.

REFERENCES


