INFLAMMATION AND OXIDATIVE STRESS IN HYPOTHYROIDISM: ADDITIVE EFFECTS ON CARDIOVASCULAR RISK

NIVEDITA NANDA1, ZACHARIAH BOBBY2* AND ABDOUNE HAMIDE3

1Department of Biochemistry, Pondicherry Institute of Medical Sciences, Puducherry, India
2Department of Biochemistry, Jawaharlal Institute of Postgraduate Medical Education and Research, Puducherry, India and
3Department of Medicine, Jawaharlal Institute of Postgraduate Medical Education and Research, Puducherry, India

Abstract: Cardiovascular disease is one of the major complications of hypothyroidism which is one of the most common endocrine disorders in India. In the present study, we have analyzed the link among oxidative stress, C reactive protein which is an inflammatory marker and the cardiovascular lipid risk factors in hypothyroid patients which has not been analyzed before. Sixty seven untreated hypothyroid patients were recruited consecutively for the study. Their ultrasensitive C reactive protein level and oxidative stress profile were measured apart from various lipid risk factors of cardiovascular disease. Ultra sensitive C reactive protein was significantly correlated with increased lipid risk factors of cardiovascular disease, thyroid stimulating hormone level and indices of oxidative stress in these patients. Low grade inflammation in hypothyroidism plausibly acts as the link between higher oxidative stress and the underlying cardiovascular risk among hypothyroid patients.

Key words: ultrasensitive C reactive protein, oxidative stress, hypothyroidism, cardiovascular risk

INTRODUCTION

Hypothyroidism is among the most common endocrine disorders in India. According to the nationwide surveys, India's burden of thyroid diseases has increased up to 42 million by the year 2000 (1). Oxidative stress (OS) has been implicated in a number of diseases such as cardiovascular diseases, neurological diseases, renal diseases,
diabetes etc (2). Presence of OS was reported in hypothyroidism too (3). In our previous study we reported the association of OS with hyperlipidemia and increased lipid risk factors of cardiovascular disease in hypothyroid patients (4). Our findings suggested OS as one of the plausible mechanisms for future atherosclerotic complications in these patients. Ultra-sensitive CRP (usCRP) is a recognized adjunctive factor for the global assessment of cardiovascular risk (5). Previous reports stating usCRP level in hypothyroidism and its role as a future risk of CHD in these patients are controversial (6, 7, 8). Hence, in this study, we assessed the link between usCRP, oxidative stress parameters and lipid risk factors of cardiovascular disease in a group of newly diagnosed untreated hypothyroid patients.

MATERIALS AND METHODS

The present study was conducted in Jawaharlal Institute of Post-graduate Medical Education and Research (JIPMER), Puducherry. In the study group, sixty seven (55 females and 12 males) newly diagnosed patients with primary hypothyroidism were recruited consecutively from the out patient department of medicine. Sixty eight gender matched healthy, euthyroid volunteers of age similar to that of study group (53 females and 5 males) were selected as controls for the study.

Subjects receiving lipid lowering drugs or antioxidant vitamin supplements, pregnant women, women on hormone replacement therapy, alcoholics, smokers, patients with hypertension and patients suffering from diseases other than hypothyroidism were excluded from the study groups. The clinician ruled out hypertension, acute and chronic infections and cardiovascular anomalies, diabetes mellitus and other endocrine diseases in the study and control group subjects. This study was approved by the research council and human ethics committee of JIPMER. Written consent was obtained from all the subjects. The patients were enrolled for the study prior to initiation of therapy.

Blood collection

Overnight fasting blood samples were collected in EDTA and serum tubes. EDTA whole blood was used for the estimation of whole blood reduced glutathione. Serum was used for the estimations of glucose and lipid profile on the same day. Remaining serum samples were refrigerated at −50°C till the estimations of thyroid profile, protein carbonyls and malondialdehyde were carried out.

Thyroid and lipid profile

Total Tri-iodothyronine (T₃) and total tetra-iodothyronine (T₄) were estimated by RIA and TSH was estimated by IRMA using kits procured from BARC (Bhaba Atomic Research Center, Mumbai, India). Estimation of serum lipid profile was carried out by using various commercial kits.

Lipid risk factors

Various lipid risk factors of atherosclerosis such as non-HDL cholesterol, total cholesterol/HDL cholesterol, triglyceride/HDL cholesterol, LDL cholesterol/HDL cholesterol.
Results

The clinical characteristics and thyroid profile are depicted in Table I. The subjects of test group had significantly higher TSH and lower T₃ and T₄ levels in comparison to the subjects of the control group. There was a significant rise in the bodyweight (BW) and BMI of the hypothyroid subjects compared to controls. The lipid profile showed a significant rise in TC, LDL-C, TG and VLDL-C in the test group (Table II). There was no difference in the level of serum HDL-C and glucose.

**Table I**: Comparison of general parameters and thyroid profile of control subjects and hypothyroid patients (mean±SD).

<table>
<thead>
<tr>
<th></th>
<th>Control (n=68)</th>
<th>Cases (n=67)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>General parameters</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>34.19±11.92</td>
<td>35.32±11.23</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>58.83±10.38</td>
<td>66.93±9.80**</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.38±3.55</td>
<td>27.98±4.05*</td>
</tr>
<tr>
<td>Fasting glucose (mg/dL)</td>
<td>76.94±12.92</td>
<td>80.43±10.51</td>
</tr>
<tr>
<td><strong>Thyroid profile</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T₃ (ng/dl)</td>
<td>127.50±28.31</td>
<td>63.90±30.15**</td>
</tr>
<tr>
<td>T₄ (mg/dl)</td>
<td>8.90±1.80</td>
<td>3.80±2.26**</td>
</tr>
<tr>
<td>TSH (µIU/ml)</td>
<td>2.24±1.10</td>
<td>64.43±36.01*</td>
</tr>
</tbody>
</table>

*P<0.01; **P<0.001, when significance was checked by Student’s t test. °P<0.001, when significance was checked by Mann Whitney-U non-parametric test.

Various coronary lipid risk factors, for the assessment of risk for atherosclerosis were calculated from fasting lipid profile (Table II). Each of these coronary lipid risk factors were found significantly higher than controls. There was a significant increase in the level of serum protein carbonyls and serum MDA in hypothyroid patients. GSH was significantly reduced in the test group whereas the activity of erythrocyte antioxidant enzyme glutathione peroxidase...
DISCUSSION

Cardiovascular risk in patients with hypothyroidism is usually attributed to an atherogenic lipid profile. However, atherosclerosis besides being a disease of lipid accumulation is also characterized by a chronic inflammatory process (12). usCRP is an independent and stable risk factor for coronary artery disease (13). Previous reports stating usCRP level in hypothyroidism and its role as a future risk of CHD in these patients are controversial (6, 7, 8). Moreover, these reports did not elucidate the plausible mechanisms behind this association. Christ et al first reported an elevated CRP and homocysteine levels in hypothyroid patients in 2003 citing it as an additional risk factor for development of CHD in hypothyroid patients. However, in 2004 Luboshitzky et al contradicted this by reporting that these two factors do not contribute significantly to increased risk for CHD in hypothyroidism (7). Similarly, in a recent study on a group of subclinical hypothyroid patients there was no elevation in CRP and other markers of CHD (8). Moreover, they found no association between these markers with elevated TSH levels. We found significant increase in serum level of usCRP and various lipid risk factors for coronary artery disease (Table II), which corroborates with the study by Christ et al. In our study there was also a strong association of usCRP with cardiovascular lipid risk factors among the hypothyroid patients which was not reported by previous studies.

Additionally we found a positive correlation of usCRP level with TSH and OS parameters such as MDA and PCO and a negative correlation with GSH which is a

TABLE II: Comparison of lipid profile, lipid risk factors, various parameters of oxidative stress and usCRP of control subjects and hypothyroid patients (means±SD).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (n=68)</th>
<th>Cases (n=67)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipid profile</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC (mg/dL)</td>
<td>174.00±31.05</td>
<td>252.05±50.50*</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>50.05±11.49</td>
<td>46.55±11.94</td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td>101.93±29.48</td>
<td>160.87±57.88*</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>110.14±38.14</td>
<td>223.16±73.75*</td>
</tr>
<tr>
<td>VLDL-C (mg/dL)</td>
<td>22.02±7.62</td>
<td>44.63±14.75*</td>
</tr>
<tr>
<td>Lipid risk factors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-HDL-C (mg/dL)</td>
<td>123.96±32.50</td>
<td>205.50±64.48*</td>
</tr>
<tr>
<td>TG/HDL-C</td>
<td>1.02±0.49</td>
<td>2.20±1.09*</td>
</tr>
<tr>
<td>TC/HDL-C</td>
<td>3.65±1.05</td>
<td>5.88±2.30*</td>
</tr>
<tr>
<td>LDL-C/HDL-C</td>
<td>2.16±0.87</td>
<td>3.83±1.92*</td>
</tr>
<tr>
<td>Atherogenic index</td>
<td>-0.05±0.18</td>
<td>0.30±0.21*</td>
</tr>
<tr>
<td>OS parameters</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood GSH (mmol/g Hb)</td>
<td>9.44±2.72</td>
<td>7.32±2.57*</td>
</tr>
<tr>
<td>Serum MDA (µM/L)</td>
<td>1.56±0.55</td>
<td>2.25±1.44*</td>
</tr>
<tr>
<td>PCO (µM/mg protein)</td>
<td>1.26±0.44</td>
<td>2.36±1.01*</td>
</tr>
<tr>
<td>Marker of inflammation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>usCRP (mg/L)</td>
<td>0.19±0.08</td>
<td>0.45±0.18*</td>
</tr>
</tbody>
</table>

P<0.001, * is used when significance was checked by Student’s t test. # when checked by Mann Whitney U non parametric test. Atherogenic index = log_{10} (TG/HDL-C).

TABLE III: Pearson correlation analyses of usCRP with TSH, OS parameters and lipid risk factors in hypothyroid cases (n=67).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>r</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSH</td>
<td>0.464</td>
<td>0.000</td>
</tr>
<tr>
<td>MDA</td>
<td>0.284</td>
<td>0.038</td>
</tr>
<tr>
<td>PCO</td>
<td>0.308</td>
<td>0.023</td>
</tr>
<tr>
<td>GSH</td>
<td>-0.526</td>
<td>0.000</td>
</tr>
<tr>
<td>Non-HDL cholesterol</td>
<td>0.493</td>
<td>0.000</td>
</tr>
<tr>
<td>TG/HDL cholesterol</td>
<td>0.355</td>
<td>0.009</td>
</tr>
<tr>
<td>TC/HDL cholesterol</td>
<td>0.498</td>
<td>0.000</td>
</tr>
<tr>
<td>LDL/C/HDL cholesterol</td>
<td>0.502</td>
<td>0.000</td>
</tr>
<tr>
<td>Atherogenic index</td>
<td>0.366</td>
<td>0.007</td>
</tr>
</tbody>
</table>

P<0.05 is considered significant.
protective antioxidant (Table III). This may indicate that the degree of inflammation increases with development of hypothyroidism (increase in TSH) and it may contribute to increased OS level with progression of this disease.

The severity of the disease as assessed by the level of TSH is another determining factor for the rise in usCRP level in our study. In a study by Gursoy et al (14), CRP level correlated with the FT4 and TSH levels of hypothyroid patients undergoing thyroid replacement therapy. However, in none of the previous reports the role of elevated OS in relation to inflammation was analyzed in hypothyroid individuals. Dardano et al in 2006 showed that acute rise in TSH level for a short term can promote low grade inflammation and oxidative stress in circulation (15) which supports our finding where TSH level was high in patients' circulation over a period of many months before being diagnosed.

Hypothyroidism is a treatable endocrine dysfunction. However, it is a disease that requires life long drug supplementation with careful monitoring of thyroid profile to avoid iatrogenic side effects especially on heart. Often the drug therapy takes long to normalize the thyroid profile. (16). In another report it was found that despite treatment in primary hypothyroidism, patients are still at increased risk of morbidity associated with various circulatory disease, ischemic heart disease, dysrhythmias and cerebrovascular diseases (17).

From our data we propose that inflammation can exacerbate OS in hypothyroid patients and both the factors can exert an additive effect on the risk of atherosclerosis and cardiovascular disease in hypothyroid patients. To the best of our knowledge, our study is the first of its kind to report an association of elevated CRP with increased OS and various lipid risk factors of CAD in hypothyroid patients. This could be vital while selecting the modified treatment protocols especially for Indian hypothyroid patients who appear to be more vulnerable to OS and inflammation. Future studies analyzing the effect of antioxidant therapy on OS parameters and inflammatory markers in hypothyroidism would further validate the present findings.

ACKNOWLEDGEMENTS

We acknowledge the research grant from Indian Council of Medical Research (ICMR) in the form of Junior and then Senior research fellowships to Dr. Nivedita Nanda and ICMR adhoc research grant to Dr. Zachariah Bobby.

REFERENCES

is independent of body mass index. Metabolism 2007; 56: 1350–1355.


