DOES HYPOTHYROIDISM PREDISPOSES PREMENOPAUSAL FEMALES TO INCREASED RISK OF ATHEROSCLEROSIS

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ABSTRACT
Background: Euthyroid state is preferred for cardiovascular system. The most common cause of cardiovascular disease is atherosclerosis. Studies have suggested anti-atherosclerotic effect of thyroid hormone but some recent clinical trials have failed to show an association between thyroid function and cardiovascular events, indicating that the relationship between thyroid hormone and atherogenesis is still not conclusive. Methods: Study was conducted on 90 premenopausal females in each group with subclinical hypothyroidism, overt hypothyroidism and healthy controls. Patients were ruled out for cardiovascular disease or any other condition which would have influenced the parameters under study. After overnight fasting, 6 mL venous blood sample was collected. hs-CRP, PAI-1, APC levels were estimated by ELISA and NO levels by modified Griess method. Results: We found that APC levels were significantly decreased and PAI-1, hs-CRP and NO levels were significantly increased in patients with overt hypothyroidism as compared to controls. APC levels were and PAI-1 were not significantly different whereas hs-CRP and NO levels were significantly increased in patients with subclinical hypothyroidism as compared to controls. Conclusion: Premenopausal females are generally considered to be at lower risk of cardiovascular disease because of protective action of estrogen but chances of cardiovascular disease due to atherosclerosis increase in females with overt hypothyroidism, which represents a potential hypercoagulable, inflammatory state with endothelial dysfunction. Females with subclinical hypothyroidism also have higher preponderance of atherosclerotic risk factors like endothelial dysfunction and inflammatory state. So, we suggest early screening of risk factors of atherosclerosis in premenopausal females with hypothyroidism.

Keywords: Hypothyroidism, cardiovascular disease, premenopausal females.

INTRODUCTION
Euthyroid state is preferred for the cardiovascular system because hypothyroidism has been associated with increased risk of cardiovascular disease. The most common cause of cardiovascular disease is atherosclerosis. Various risk factors for development of atherosclerosis include hypercholesterolemia, cigarette smoking, hypertension and diabetes mellitus. Elevation of hs-CRP and an increase in plasminogen activator inhibitor-1 (PAI-1) levels are emerging as new risk factors for atherosclerotic cardiovascular diseases.

CRP is an acute-phase protein which reflects overall atherosclerotic burden and extravascular inflammation which potentiates atherosclerosis or its complications. CRP measured by high-sensitivity assay is useful in the predicting cardiovascular risk. Study by Christ-Crain showed raised levels of CRP level in patients with hypothyroidism as compared to non-hypothyroid group. Thyroid dysfunction influences endothelial function. Studies by Taddei et al and Papaioannou et al showed that replacement of thyroid hormone improves endothelial function in patients with hypothyroidism.
Thyroid hormones; T₄ and T₃ have cardiovascular effects, probably through the regulation of circulating clotting proteins and fibrinolytic activity⁹. In patients with hypothyroidism increased levels of PAI-1 have been observed by Erem and colleagues⁺,¹¹. Protein C is one of the most important physiological anticoagulant components. After activation by thrombin–thrombomodulin complexes on platelets and endothelial cells, protein C is converted to activated protein C (APC), which inactivates factors Va and VIIIa in the presence of protein S and phospholipids, which are the main constituents of the plasma membrane of platelets and endothelial cells. This means that APC acts as an anticoagulant at just the sites of thrombus generation. Activated protein C also inactivates type 1 plasminogen activator inhibitor (PAI-1), thereby promoting fibrinolysis¹².

Studies have suggested anti-atherosclerotic effect of thyroid hormone and an association of thyroid dysfunction with atherogenesis, but some recent clinical trials have failed to show an association between thyroid function and cardiovascular events, indicating that the relationship between thyroid hormone and atherogenesis is still not conclusive. Moreover, premenopausal females are at low risk of atherosclerosis because of protective effect of estrogen. Does the protective effect of estrogen ameliorate the chances of atherosclerosis in premenopausal females in not known. So, we planned to evaluate whether hypothyroidism predisposes premenopausal females to increased risk of atherosclerosis.

**MATERIALS AND METHODS**

The study was conducted after obtaining ethical clearance from institute. We enrolled 270 premenopausal females of age group 20-40 years referred to hormone laboratory in a tertiary care hospital of northern India. After informed consent thyroid function test was performed and females were further subdivided into 3 groups: 90 premenopausal females with subclinical hypothyroidism, 90 premenopausal females with overt hypothyroidism and 90 age matched premenopausal healthy controls. All females were ruled out for any history suggestive of cardiovascular disease, blood coagulation disorders or any other inflammatory condition, drug intake which would have influenced the parameters under study. After overnight fasting, 6 mL venous blood sample was collected. Serum was separated and stored at -40°C until batch analysis.

**METHODOLOGY**

Serum TSH, fT4 and fT3 levels were assayed using fully automated chemiluminescent immunoassay Analyzer Access 2 by Beckman and Coulter (USA). Reference intervals of TSH is 0.34-5.6µIU/mL, fT3 2.5-3.9µg/dL, and serum fT4 0.6-1.12 ng/dL. Females with TSH >0.34 to 5.6µIU/mL and fT3 and fT4 levels within the reference range were considered as euthyroid. Patients with TSH levels >5.7-9.9 µIU/mL and normal fT3 and fT4 levels were enrolled into subclinical hypothyroid group and patients with TSH levels >10 µIU/mL, fT3 levels < 2.5-µg/mL and fT4 levels < 0.6 ng/dL were enrolled into overt hypothyroidism group. Nitric oxide (NO) was estimated by modified Griess method. PAI-1, activated protein C, hs-CRP levels were estimated by ELISA using commercially available kit.

**RESULTS**

<table>
<thead>
<tr>
<th>Table 1: Baseline characteristics of the subjects</th>
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<tbody>
<tr>
<td><strong>Age</strong> (years)</td>
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<tr>
<td>-----------------</td>
</tr>
<tr>
<td>Overt Hypothyroidism</td>
</tr>
<tr>
<td>Subclinical hypothyroidism</td>
</tr>
<tr>
<td>Controls</td>
</tr>
<tr>
<td><strong>p value</strong></td>
</tr>
<tr>
<td><strong>p value^</strong></td>
</tr>
</tbody>
</table>

# p value between overt hypothyroid patients and controls
^ p value between subclinical hypothyroid patients and controls
* p value <0.05; significant as compared to controls
** p value <0.001; highly significant as compared to controls
TABLE 2: Markers of atherosclerosis in females with hypothyroidism as compared to controls (mean ± SD)

<table>
<thead>
<tr>
<th></th>
<th>Overt Hypothyroidism</th>
<th>Subclinical Hypothyroidism</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSH</td>
<td>11.66 ± 7.3*</td>
<td>8.12 ± 0.05§</td>
<td>3.56 ± 0.99</td>
</tr>
<tr>
<td>Free T3</td>
<td>1.99 ± 0.64*</td>
<td>3.58 ± 2.69</td>
<td>3.55 ± 0.99</td>
</tr>
<tr>
<td>Free T4</td>
<td>0.53 ± 0.29*</td>
<td>0.81 ± 0.89</td>
<td>0.84 ± 0.16</td>
</tr>
<tr>
<td>PAI-1</td>
<td>46.58 ± 17.23*</td>
<td>31.26 ± 9.90</td>
<td>30.15 ± 8.2</td>
</tr>
<tr>
<td>Protein C</td>
<td>2.83 ± 1.79*</td>
<td>5.34 ± 1.10</td>
<td>5.24 ± 1.51</td>
</tr>
<tr>
<td>NO</td>
<td>29.45 ± 8.47*</td>
<td>27.64 ± 6.44*</td>
<td>11.62 ± 9.99</td>
</tr>
<tr>
<td>hs CRP</td>
<td>10.03 ± 4.04*</td>
<td>7.30 ± 1.67**</td>
<td>5.53 ± 4.12</td>
</tr>
</tbody>
</table>

*p value <0.001; highly significant as compared to controls
**p value <0.05; significant as compared to controls

DISCUSSION
Cardiovascular system is very sensitive to thyroid function. Hypothyroidism can cause or accelerate cardiovascular diseases. Thyroid hormone has been suggested to have anti-atherosclerotic effect. Accumulating evidence from studies suggest that maintaining the euthyroid state is beneficial to prevent the progression of atherosclerosis. Endothelial dysfunction, inflammation and increased coagulation all play a crucial role in the atherothrombotic process. Endothelial dysfunction is generally accepted as an early step of the atherosclerosis. A reduction of NO availability has been suggested as the mechanism of endothelial dysfunction in patients with hypothyroidism. In our study we found decreased levels of NO in patients with overt as well as subclinical hypothyroidism as compared to controls. NO along with inhibition of initiation and progression of atherosclerotic plaques, also inhibits the inflammatory and prothrombotic response. Thus, low levels of NO may lead to prothrombotic state within atherosclerotic lesions and a higher cardiovascular risk.

CRP is a biomarker of atherosclerosis. In our study we found significantly high hs- CRP levels in patients with subclinical as well as overt hypothyroidism as compared to controls. This suggests patients with hypothyroidism are at high risk of future cardiovascular events because of increased burden of atherosclerosis. C-reactive protein is not only a marker of the atherosclerotic inflammatory process but it also plays an active role in the pathogenesis of atherosclerosis. CRP increases propensity to atherosclerotic lesion by inducing the production of cell adhesion molecules such as endothelin-1, it also induces expression of tissue factor in monocytes, oxidation of LDL-cholesterol and compliment activation. Absorption of LDL by macrophages and recruitment of monocytes to the arterial wall is also mediated by CRP thus contributing towards pathogenesis of atherosclerosis.

The influence of thyroid hormone on the coagulation-fibrinolytic system is mainly mediated by the interaction between hormone and its receptors. Fibtrinolytic system plays an important role in eliminating fibrin clots, thereby decreasing propensity to form thrombi. PAI-1 is the main inhibitor of the fibrinolytic system. Increase in PAI-1 levels would increase the chances of thrombus formation. PAI-1 also influences the development of atherosclerosis by affecting matrix degradation, smooth muscle cell migration and angiogenesis. In our study we found that Serum PAI-1 levels were significantly increased in females with overt hypothyroidism as compared to controls but levels were not significantly different in females with subclinical hypothyroidism and controls.

APC levels were significantly decreased in females with overt hypothyroidism as compared to controls and not significantly different in females with subclinical hypothyroidism as compared to controls.

APC plays a critical role in down-regulating the clotting cascade, low APC levels would increase the chances of clot formation. Down-regulation of protein C activity would also result in neutrophil adhesion to the endothelium increasing inflammation. Increased inflammation and clot formation in these patients thereby, would potentiate atherosclerotic process.

In conclusion, overt hypothyroidism represents a potential hypercoagulable, inflammatory state with endothelial dysfunction, which might augment the process of atherosclerosis and its complications. Females with subclinical hypothyroidism have higher preponderance of atherosclerotic risk factors like endothelial dysfunction and inflammatory state. Also, patients had more atherogenic lipid profile as compared to healthy controls suggesting patients are at increased risk of cardiovascular disease.

Premenopausal females generally considered to be at lower risk of cardiovascular disease.
because of protective action of estrogen but chances of atherosclerosis increase in females with hypothyroidism so, we suggest early screening of risk factors of atherosclerosis in these females.

Given the high prevalence of hypothyroidism, this question needs to be addressed in randomized controlled trial with larger sample size.

REFERENCES

19. Ridker PM. C-reactive protein and the prediction of cardiovascular events among those at intermediate risk: moving an inflammatory hypothesis


