Relation between carotid intima media thickness and vitamin D in hypertension.

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Abstract

Purpose: Subclinical atherosclerosis defined as endothelial dysfunction, coronary calcification, and increased carotid intima media thickness is related to cardiovascular events independent from blood pressure. There are data showing possible relation between low vitamin D levels and subclinical atherosclerosis. In this study we aimed to find out the relation between vitamin D level and intima media thickness and the presence of atherosclerotic plaques.

Materials and methods: Fifteen male and 148 female subjects with essential hypertension aged between 30-76 were included to the study. Intima media thickness was measured and the presence of plaque was evaluated by real time B mode ultrasonography (MyLab 70 XVG, Esaote SpA, Genoa, Italy) using 4.0-13.0 MHz linear probe. Serum parathormone was measured by chemiluminiscence immunoassay and 25 OH vitamin D level by radioimmunounassay.

Results: Eighty patients (49%) had plaques. Seventy three patients (45%) had severe vitamin D deficiency defined as level below 10 ng/ml and 64 (39%) had insufficiency, (10-20 ng/ml). Vitamin D levels were similar in plaque +ve and –ve groups (p=0.44). Intima media thickness was positively correlated with age (r=0.399, p=0.0001), fasting glucose (r=0.165, p=0.036), and Hemoglobin A1c (r=0.384, p=0.002). There was no correlation between intima media thickness and vitamin D level (p=0.75) and LDL (p=0.581).

Conclusion: We did not find any relation between vitamin D level and intima media thickness in hypertensive subjects. The lack of significance may be due to high prevalence of vitamin D deficiency.

Keywords: Vitamin D, Hypertension, Subclinical atherosclerosis.

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Introduction

High blood pressure is associated with increased risk of cerebrovascular disease and coronary artery disease [1]. Hypertension leads to arterial and arteriolar sclerosis via endothelial cell changes and remodelling of smooth muscle cells underlining endothelium [2]. As a result target organ damage ensues. Subclinical atherosclerosis is defined as endothelial dysfunction, coronary calcification, and increased carotid intima media thickness (IMT).IMT is related to cardiovascular events independent from conventional vascular risk factors including blood pressure [3].

Increased IMT and plaque formation are biologically and genetically distinct entities [4]. IMT is mainly caused by hypertensive medial hypertrophy and weakly associated with traditional coronary risk factors. IMT is more predictive of stroke than of myocardial infarction. On the contrary plaque formation is more strongly associated with traditional risk factors and is more predictive of myocardial infarction than of stroke [5]. It was shown that after adjustment for age, gender, blood pressure, cholesterol, smoking, diabetes mellitus (DM), and treatment of lipids and blood pressure, patients in the top quartile of plaque area had 3.5 times the risk of those in the lowest quartile [5].

Plaques grow along the carotid arteries 2-4 times faster than they thicken [4]. Both calcified and non-calcified plaques are independent predictors of vascular disease at all ages. Measurement of IMT is a non-invasive, simple, reliable, and safe method in detecting subclinical atherosclerosis [3]. IMT is measured in vascular segments free of plaque [4].

Beyond established cardiovascular risk factors epidemiologic studies indicate that vitamin D deficiency is an independent risk factor for cardiovascular events [6-8].

Similarly, there are data showing possible relation between low vitamin D levels and subclinical atherosclerosis [6,9]. Vitamin D (vitamin D) receptors are expressed by endothelial cells and vascular smooth muscle cells [9].
Low vit D level also activates renin angiotensin system (RAS) that is upregulated in essential hypertension [6,10]. Although some data favours the role of vitamin D in subclinical atherosclerosis, the relation has not been established yet.

In this study we aimed to find out the relation between 25 hydroxy (OH) vitamin D level and subclinical atherosclerosis (IMT and atherosclerotic plaques) in hypertensive patients.

**Materials and Methods**

A total number of 176 patients (15 male and 161 female subjects) with essential hypertension aged between 30-76 were included to the study. The patients were already on hypertensive medication.

Secondary hypertension was excluded in case of clinical suspicion (such as age and resistant hypertension). Neither of patients were hypokalemic even some of them were on diuretic therapy. None of the patients were on steroid medication. None of the patients were on parathyroid adenoma. None of the patients were on diuretic therapy. None of the patients were on any known or suspected metabolic bone disease except osteoporosis.

History of coronary artery disease (established by angiography, nuclear scanning, suspicious ECG, and angina pectoris), cerebrovascular disease, transient ischemic attack, peripheral artery disease, chronic liver disease, collagen vascular disease, and type 1 DM were other exclusion criteria.

Serum lipid levels, urea, creatinine, sodium, potassium, alanine aminotransferase (ALT) values, total albumin, calcium, phosphorus, and haemoglobin A1c measured by photometric method using autoanalyzer (Siemens, Advia 1800) within preceding year were documented. After an overnight fast venous blood sample was studied for serum PTH by chemiluminiscence immunoassay (Siemens, Advia 1800, Centour XP). TSH and 25 OH vitamin D were also studied using chemiluminiscence immunoassay (Siemens, Advia 1800).

**Corrected calcium level was calculated according to the formula**

\[ 0.8\times (4.0\text{-patient’s albumin}) + \text{patient’s serum calcium} \]

The subjects with corrected calcium above normal level, any biochemical and clinical suspicion of parathyroid adenoma were excluded.

Parathormone (PTH) begins to rise as glomerular filtration rate (GFR) falls below 60 mL/min. Therefore GFR was calculated according to the MDRD formula [11]. GFR lower than 30 mL/min were excluded.

IMT was measured and the presence of plaque was evaluated by real time B mode ultrasonography (MyLab 70 XVG, Esaote SpA, Genoa, Italy) using 4.0-13.0 MHz linear probe. All ultrasonographic assessment was done by the same experienced radiologist who was blind to all data but hypertension. Standard procedure was supine position, taking many longitudinal and transverse planes of carotid arteries till getting the best image. The measurement of common carotid artery and internal carotid artery was done 10 mm proximal and 10 mm distal to the bifurcation free of plaque respectively. The distance from the media-adventitia interface to lumen-intima was measured. The arithmetic mean value of right and left measurements was denoted as mean IMT. Atherosclerotic plaque was defined as intima-media thickening over 1 mm or doubles that of the adjacent vascular segment [12].

The study protocol was approved by local ethics committee and was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

**Statistics**

SPSS version 10.0 was used for statistical analysis. All parametric variables showing normal distribution excluding diabetes duration, hypertension duration, fasting glucose, TSH, 25 OH vitamin D, HDL, CIMT were evaluated with Student’s t test using. The other variables not showing normal distribution were evaluated with Mann Whitney U test. Correlation analysis between CIMT and other variables was done using Pearson correlation analysis. The parametric values were given as mean and standard deviation. Chi square test was done for nonparametric variables. P below 0.05 was considered statistically significant.

**Results**

Eighty seven patients (49%) had plaques. There was not a side predilection for plaque formation (25 on the right side, 30 on the left, and 32 bilateral). The laboratory data and characteristics of plaque +ve and –ve patients are summarized in Table 1.

**Table 1. Demographic data and laboratory values of patients classified according to the presence of plaques.**

<table>
<thead>
<tr>
<th></th>
<th>Plaque (+)</th>
<th>Plaque (-)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>62.07 ± 7.69</td>
<td>55.17 ± 7.99</td>
<td>0.0001</td>
</tr>
<tr>
<td>Gender (F/M)</td>
<td>81/6</td>
<td>80/9</td>
<td>0.44</td>
</tr>
<tr>
<td>Duration of HT (years)</td>
<td>9.16 ± 7.22</td>
<td>6.51 ± 5.84</td>
<td>0.008</td>
</tr>
<tr>
<td>DM (n)</td>
<td>31</td>
<td>23</td>
<td>0.15</td>
</tr>
<tr>
<td>Duration of DM (years)</td>
<td>7.41 ± 6.73</td>
<td>6.17 ± 5.04</td>
<td>0.46</td>
</tr>
<tr>
<td>History of smoking (n)</td>
<td>13</td>
<td>26</td>
<td>0.04</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>32.31 ± 5.10</td>
<td>33.12 ± 6.84</td>
<td>0.37</td>
</tr>
<tr>
<td>Obesity (BMI ≥ 30 kg/m²) (n)</td>
<td>58</td>
<td>54</td>
<td>0.46</td>
</tr>
<tr>
<td>Fasting glucose (mg/dL)</td>
<td>117.87 ± 57.04</td>
<td>114.84 ± 69.49</td>
<td>0.89</td>
</tr>
<tr>
<td>HbA1c (%) (n=35)</td>
<td>7.67 ± 1.83</td>
<td>7.88 ± 2.29</td>
<td>0.63</td>
</tr>
<tr>
<td>Cr (mg/dL)</td>
<td>0.82 ± 0.21</td>
<td>0.78 ± 0.17</td>
<td>0.13</td>
</tr>
<tr>
<td>K (meq/L)</td>
<td>4.66 ± 0.46</td>
<td>4.50 ± 0.33</td>
<td>0.04</td>
</tr>
<tr>
<td>Corrected Ca (mg/dL)</td>
<td>9.37 ± 0.31</td>
<td>9.38 ± 0.33</td>
<td>0.90</td>
</tr>
<tr>
<td>P (mg/dL)</td>
<td>3.49 ± 0.44</td>
<td>3.51 ± 0.54</td>
<td>0.94</td>
</tr>
</tbody>
</table>
Plaque +ve patients were older than plaque –ve ones (p=0.0001). Gender distribution was similar in both groups (female: 81 in plaque +ve and 80 in plaque-ve group; p=0.44). Plaque +ve patients had longer duration of hypertension (p=0.008). The number of patients receiving ACEI/ARB, statin, calcium channel blocker, diuretic, and calcium-vitamin D were not different between plaque +ve and –ve groups.

There was no statistically significant difference between plaque +ve and –ve groups in terms of frequency of DM (p=0.15) and mean duration of DM (p=0.46). Vitamin D levels were similar in both groups (p=0.41). Plaque +ve patients had higher CIMT as expected (p=0.0001).

Mean PTH, 25 OH D vitamin, ALP, calcium corrected for albumin, phosphorus, fasting glucose, haemoglobin A1c, LDL, HDL, and triglyceride levels were similar between plaque +ve and –ve groups. Although mean creatinine was similar between two groups (p=0.13), MDRD was significantly lower in plaque +ve group (p=0.01). Plaque +ve patients had higher TSH level (p=0.04) even when extreme values were deleted and comparative statistics were redone (p=0.05).

Sixty six patients had GFR above 90 ml/min, 88 had between 60-89 ml/min, and 22 had between 31- 59 ml/min. IMT and vitamin D level was similar between MDRD stages.

IMT was positively correlated with age (r=0.399, p=0.0001), fasting glucose (r=0.165, p=0.036), HbA1c (r=0.384, p=0.002), and negatively correlated with MDRD (r=0.148, p=0.05) (Table 2). There was no correlation between vitamin D level (p=0.75) and LDL (p=0.581).

**Table 2. Correlation analysis between IMT and demographic and laboratory data.**

| ALP (U/L) | 82.23 ± 22.25 | 86.01 ± 33.76 | 0.35 |
| TSH (mU/L) | 4.14 ± 10.05 | 3.74 ± 3.40 | 0.04 |
| TSH (mU/L) | 2.74 ± 3.40 | 1.92 ± 1.56 | 0.05 |
| PTH (pg/mL) | 72.15 ± 34.57 | 51.49 ± 11.58 | 0.60 |
| 25 OH D vit (ng/mL) | 13.34 ± 6.87 | 163.92 ± 64.47 | 0.41 |
| LDL (mg/dL) | 128.20 ± 34.45 | 51.49 ± 11.58 | 0.60 |
| HDL (mg/dL) | 51.49 ± 11.58 | 163.92 ± 64.47 | 0.74 |
| Vit D sufficiency** (n) | 79.61 ± 20.03 | 13 | 0.01 |
| Mean IMT (mm) | 0.73 ± 0.14 | 0.64 ± 0.11 | 0.0001 |

"Extreme values were deleted "25 OH D vit>20 ng/mL.

DM: Diabetes Mellitus; HT: Hypertension; BMI: Body Mass Index; HbA1c: Haemoglobin A1c; Cr: Creatinine; K: Potassium; Ca: Calcium; P: Phosphorus; ALP: Alkaline Phosphatase; TSH: Thyroid Stimulating Hormone; PTH: Parathormone; LDL: Low Density Lipoprotein; HDL: High Density Lipoprotein; 25 OH D vit: 25 Hydroxy Vitamin D3; MDRD: Glomerular Filtration Rate Calculated According to Modification of Diet in Renal Disease Study; IMT: Intima Media Thickness of Carotid Artery.

Vitamin D deficient patients had significantly higher A1c level when compared to vitamin D competent ones (p=0.04). Vitamin D competent patients had lower BMI compared to Vitamin D deficient ones (p=0.028). Albumin levels were significantly lower in Vitamin D deficient patients in comparison to Vitamin D insufficient (p=0.039) and vitamin D competent ones (p=0.022). But total calcium level corrected for albumin showed no difference between D vitamin groups.

**Discussion**

Subclinical atherosclerosis documented by increased IMT predicts future cardiovascular events [3]. Hypertension, smoking, high LDL cholesterol, and impaired glucose
metabolism are conventional cardiovascular risk factors. IMT increases with age [13,14].

In our study IMT was positively correlated with age and plaque +ve patients were older than plaque-ve ones. The plaque +ve subjects had longer duration of hypertension (p=0.008). Our data supports the literature showing positive relation between duration of hypertension in years and increased IMT [15-17].

High LDL and triglyceride and low HDL contribute to plaque formation. In ARIC study it was shown that IMT was strongly associated with atherogenic lipids, smoking, and hypertension [14].

In our study lipid fraction levels were similar between plaque +ve and –ve groups. The number of patients on statin therapy was not different between plaque +ve and –ve groups (n=0.26). Neither IMT was correlated with LDL level (p=0.581). This may indicate that there are factors other than lipid levels (residual risk factors) and statin usage affecting plaque formation. There was no correlation between vitamin D level (p=0.75) and LDL (p=0.581).

Smoking is a well-established contributing factor in atherosclerotic vascular disease. In our study both current and past smokers constituted 22% of subjects. Plaque +ve patients had lower frequency smoking (both current and past smoking). This finding is also contradictory to ARIC study [14].

IMT also increases in diabetes mellitus [18]. In our study IMT was positively correlated with fasting glucose (r=0.165, p=0.036) and HbA1c (r=0.384, p=0.002) (Table 2). But plaque +ve and –ve groups were not different in terms of presence and duration of DM (p=0.15 and p=0.46, respectively). We also failed to find statistically significant difference in terms of fasting glucose and A1c (p=0.63 and p=0.89, respectively). Although DM is a contributing factor in plaque formation, we did not find any relation between DM and plaque formation.

Even extreme TSH values were extracted plaque +ve patients had higher TSH values. IMT may decrease with LT4 replacement in subclinical hypothyroidism defined as normal TSH level with normal free T4 and T3 levels [19].

The decrement was observed in patients with no established cardiovascular risk factors under age 55 and accompanied improvement of lipid profile. On the other hand, vascular smooth muscle cells express iodothyronine deiodinase type II. Effect of TSH may be beyond improvement of the lipid profile. The increase in IMT reaches significant values at TSH levels above 10 mIU/l [20].

Impaired lipid profile increased blood pressure accompanies subclinical hypothyroidism and may be responsible for increased IMT [20].

Vitamin D deficiency is defined as levels below 20 ng/ml though optimal level is over 30-32 ng/ml for many of the physiologic functions [21]. We detected high frequency of vitamin D deficiency. Only 20.5% of the patients were vitamin D competent (>20 ng/ml). In most of the cohort studies the frequency of vitamin D deficiency is 52-77% when the cut off level is 30 ng/ml [10]. The prevalence of vitamin D deficiency is about 44-60% in Turkey (Vitamin D status and bone mineral density of veiled and unveiled Turkish women [22-24].

In a study done in 254 patients in South part of Turkey showing low socioeconomic status, the frequency of vitamin D deficiency was 94% when 20 ng/ml was taken as cut off level (unpublished data, poster presentation in Turkish Endocrinology and Metabolic Diseases Congress, 2010, Antalya). Also a large scale (n=9560) study evaluating vitamin D status in Turkey, high prevalence (93%) of vitamin D deficiency (<20 ng/ml) was compatible with our results [25].

Mean BMI was over 30 kg/m² in plaque +ve and –ve groups. Vit D competent patients had lower BMI compared to Vitamin D deficient ones (p=0.028). Published data indicate vitamin D is lower in obese patients. It is suggested that lipid soluble vitamin D is deposited in adipose tissue. The reason of low vitamin D levels in obese patients may be beyond this simple explanation. Gap junctional communications between osteoblastic progenitors may cause mesenchymal switch to adipogenesis [26]. Animal studies show vitamin D inhibits adipogenesis and induces osteoblastogenesis [26].

In the literature both high and low levels of 25 OH vitamin D are associated with coronary artery disease [6]. Vitamin D may suppress vascular smooth muscle cell proliferation by inhibiting cyclin-dependent kinase-2 activity [6]. High vitamin D level is blamed for arterial calcification. We did not find any relation neither between vitamin D level and IMT nor between vitamin D and plaque formation in hypertensive subjects. The lack of significance may be due to high prevalence of vitamin D deficiency. In a paper published from Turkey, no relation was detected between vitamin D level and plaque type or IMT in hypertensive patients. But vitamin D levels were much higher than ours [27]. In another study an inverse relation was found between vitamin D level and IMT in hypertensive subjects. The modest but significant effect of vitamin D on systolic blood pressure was mediated by increased intima media thickness and endothelial dysfunction [28].

In conclusion we suggest that increased IMT and plaque formation may differ regarding pathogenesis. Only age and decreased MDRD are common risk factors for both increased IMT and plaque formation. While duration of hypertensive injury and smoking facilitates plaque formation, impaired glucose metabolism adversely affects IMT. Serum vitamin D levels do not appear to play a role in subclinical atherosclerosis.

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