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Serum Heart-Type Fatty Acid-Binding Protein Levels in Patients with Overt Hypothyroidism

Aşikar Hipotiroidili Hastalarda Serum Kalp Tipi Yağ Asidi Bağlayıcı Protein Düzeyleri

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Abstract

Purpose: Overt hypothyroidism affects mostly women with an increasing prevalence with age. Hypothyroidism is associated with accelerated atherosclerotic cardiovascular diseases possibly caused by the higher incidence of hyperlipidemia, insulin resistance, and hypertension. Heart-type fatty acid-binding protein (H-FABP) is specific for cardiomyocytes and a sensitive marker of myocardial injury. The purpose of this study was examining the effect of hypothyroidism on H-FABP levels and carotid artery intima-media thickness (CIMT).

Material and Method: We measured serum H-FABP levels in 33 patients with overt hypothyroidism and age, gender, and body mass index-matched 39 control subjects. The patients were newly diagnosed with Hashimoto's thyroiditis. All participants underwent high-resolution B-mode ultrasonography for the measurement of CIMT.

Results: There was no significant difference in serum levels of H-FABP between the patient group and controls (1515.87±2143.0 pg/mL vs. 953.0±416.0 pg/mL, respectively; p=0.15). CIMT level was significantly higher in the patient group than in the control group (0.53±0.08 mm vs. 0.48±0.05 mm; p=0.02). However, the homeostasis model assessment of insulin resistance and fasting insulin levels did not differ between the two groups.

Discussion: Based on the results of this study, we assume that H-FABP is not a useful marker in detecting preclinical atherosclerosis in patients with overt hypothyroidism associated with Hashimoto's thyroiditis, however, CIMT might be a useful marker in detecting early atherosclerosis.

Keywords: Carotid artery intima-media thickness, heart-type fatty acid-binding protein, coronary artery disease, atherosclerosis, hypothyroidism

Öz

Amaç: Aşikar hipotiroidi sıklıkla kadınlarda görülmekte ve yaşla birlikte prevalansı artmaktadır. Hipotiroidi, ilişkili artmış hiperlipidemi, insülin direnci ve hipertansiyon insidansına bağlı artmış aterosklerotik kardiyovasküler hastalıklar ile ilişkilidir. Kalp-tipi yağ asidi bağlayıcı protein (H-FABP) kardiyomiyositlere özgü olup kalp kası hasarının hassas bir belirtecidir. Bu çalışmanın amacı, hipotiroidinin H-FABP düzeylerine ve karotis arter intima-media kalınlığı (KİMK) üzerine etkisini incelemektir.

Gereç ve Yöntem: Serum H-FABP, aşikar hipotiroidi tanısı alan 33 hastada ve yaş, cinsiyet, ve vücut kitle indeksi uyumlu 39 kontrol olgusunda ölçüldü ve tüm olguların yüksek çözünürlüklü B-mod ultrasonografi ile KİMK ölçümleri yapıldı.

Bulgular: Kontrol ile hasta grubunda H-FABP serum düzeyleri açısından anlamlı derecede farklı bulunmamıştır (sırasıyla; 953,0±416,0 pg/mL, 1515,87±2143,0 pg/mL, p=0,15). KİMK, kontrol grubuna göre hasta grubunda anlamlı olarak yüksek bulundu (sırasıyla; 0,48±0,05 mm, 0,53±0,08 mm, p=0,02). Ancak, insülin direncinin homeostaz modeli değerlendirmesi ve açlık insülin düzeyleri açısından iki grup arasında fark yoktu.

Tartışma: Bu çalışmanın sonuçlarına göre Hashimoto tiroiditi ile ilişkili aşikar hipotiroidi hastalarında preklinik ateroskleroz tespitinde H-FABP yararlı bir belirteç değildir. Ancak KİMK erken ateroskleroz tespitinde yararlı olabilir.

Anahtar kelimeler: Karotis arter intima-media kalınlığı, kalp tipi serbest yağ asidi bağlayıcı protein, koroner arter hastalığı, ateroskleroz, hipotiroidizm

Introduction

Overt hypothyroidism affects approximately 3% of the adult female and 1% of the adult male population and its prevalence increases by age (1,2). It is associated with accelerated atherosclerosis and coronary artery disease (3,4,5,6), which indicates a risk of myocardial infarction (MI) probably caused by hypercholesterolemia, hypertension, and insulin resistance (7). The most clinically important effect of thyroxine (T4) deficiency on lipoprotein metabolism is the elevation of circulating

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low-density lipoprotein (LDL) cholesterol, hypertriglyceridemia, and impairment of fatty acid mobilization (8).

Heart-type fatty acid-binding protein (H-FABP) is a low molecular weight cytoplasmic, non-enzymatic protein that transports long-chain fatty acids in the cytosol of cardiomyocytes. When the myocardium is injured, H-FABP is rapidly released in large amounts into the circulation (9). In addition, it has also been used as an early, sensitive diagnostic marker for diseases that cause myocardial damage [i.e., acute coronary syndromes (ACS)]. Furthermore, it has been demonstrated that the serum level of H-FABP is increased in patients with advanced heart failure, hypertrophic and dilated cardiomyopathy, and pulmonary embolism indicating ongoing myocardial damage (10,11,12). Carotid artery intima-media thickness (CIMT) is a valuable tool for early diagnosis of preclinical atherosclerosis and associated with major cardiovascular risk factors (13).

In this study, we tested whether H-FABP levels increase in patients with overt hypothyroidism and evaluated the association between its levels and subclinical atherosclerosis determined by CIMT level.

Materials and Methods

This study was performed in 33 patients with overt hypothyroidism associated with Hashimoto's thyroiditis (31 women and 2 men) and 39 age-, gender-, and body mass index (BMI)-matched control subjects. Overt hypothyroidism was diagnosed by a high thyroid-stimulating hormone (TSH) (TSH>10 mIU/L) and low free T4 (fT4) (<0.89 ng/dL) concentration. After overnight fasting, blood samples were taken from all the subjects to check the glucose, insulin, fT4, TSH, LDL-cholesterol, high-density lipoprotein (HDL) cholesterol, triglyceride, total cholesterol, and H-FABP levels. Weight, height, waist, and hip circumferences (waist: midway between the lower rib margin and the iliac crest, hip: widest circumference over the great trochanters) were also measured, and the BMI and homeostasis model assessment of insulin resistance (HOMA-IR) were calculated. In addition, all patients underwent high-resolution B-mode ultrasonography for the measurement of CIMT. The same investigator carried out all the scans and imaging studies. Furthermore, all the study participants were interviewed using a standard questionnaire that included information regarding demographic characteristics, concomitant disease, use of medications that could affect H-FABP levels, and smoking history. Afterwards, the patients underwent a physical examination, and those with a history of ACS, pulmonary embolism, stroke, and heart failure along with those with immunological and renal diseases were excluded from the study. The control group was composed of volunteers who had no history of ACS, heart failure, cardiomyopathy, pulmonary embolism, renal diseases, immunological diseases, and diabetes mellitus. The study protocol was approved by the Local Ethics Committee and all patients gave their informed consent to participate in the study.

Serum Assays

Serum TSH and fT4 levels were measured with chemiluminescence assay (Advia Centaur, Siemens Healthcare Diagnostics, USA). The levels of total cholesterol, HDL-cholesterol and triglyceride were measured with enzymatic colorimetric assays by spectrophotometry (BioSystems S.A., Barcelona, Spain). The LDL-cholesterol level was calculated using the Friedewald formula. Glucose was measured by glucose oxidase (Advia 2400, Siemens Healthcare Diagnostics, USA), and insulin by non-competitive chemiluminescence (Advia XP, Siemens Healthcare Diagnostics, USA).

Heart-type Fatty Acid-Binding Protein

The H-FABP measurements were performed with the Epoch micro-volume spectrophotometer system (BioTek, Inc., Winooski, VT, USA) using a commercially available enzyme-linked immunosorbent assay ELISA kit (Hycult Biotech, Uden, The Netherlands). The assay range of the H-FABP ELISA kit was 102-25.000 pg/mL, and the measurements were calculated at the same time during the same experiment.

The ready-to-use solid-phase human H-FABP ELISA is based on the sandwich principle. Samples and standards are incubated together with peroxidase-conjugated secondary antibody in microtiter wells coated with antibodies that recognize human H-FABP. During incubation, the human H-FABP is captured by the solid bound antibody, and the secondary antibodies then bind to the captured human H-FABP. Next, the peroxidase-conjugated antibody reacts with the substrate tetramethyl benzidine, and this can only be stopped with the addition of oxalic acid. The absorbance at 450 nm is measured with a spectrophotometer.

Statistical Analysis

Statistical analyses were performed using Predictive Analysis Software version 18.0 for Windows, and mean, standard deviation, median, minimum and maximum were used for the numerical variables; frequency and percentage were used for the categorical variables as descriptive statistics. Comparisons between the groups for the categorical variables were done by chi-square test, and a t-test was carried out to determine the differences among the groups for the normally distributed data. For non-normally distributed data, the Mann-Whitney U test was performed for comparisons between the two groups. Additionally, to measure the association between the variables that were not normally distributed, Spearman's rho coefficient was calculated. A p value of less than 0.05 was considered statistically significant.

Results

The demographic characteristics of the study participants are shown in Table 1. The serum fT4 levels were significantly higher in the control subjects (1.02±0.18 ng/dL) than in the patient group (0.503±0.23 ng/dL) ($p<0.001$), but the serum TSH levels were significantly elevated in hypothyroid subjects compared with the controls (70.40±55.94 mIU/L vs. 2.10±1.52 mIU/L) ($p<0.001$). The frequency of smoking was also significantly higher in the patient group ($p<0.001$). However, the serum H-FABP levels were similar between the patient and control groups (1515.87±2143.00 pg/mL and 953.00±416.00 pg/mL, respectively; $p=0.15$). In addition, the serum total cholesterol levels were significantly higher in the patients than in the controls (220.00±73.67 mg/dL vs. 191.00±35.73 mg/dL, respectively; $p=0.03$), but there were no statistically significant differences in LDL-cholesterol, HDL-cholesterol, and triglyceride levels between the two groups. The CIMT was significantly higher in the patient group than in the control group (0.53±0.08 mm vs. 0.48±0.05 mm, respectively; $p<0.02$), however, the HOMA-IR and fasting insulin levels did not differ between the two groups.

The H-FABP levels were not correlated with the total cholesterol, HDL-cholesterol, triglyceride, TSH, insulin, and HOMA-IR levels. Additionally they were not associated with age, smoking history, waist circumference or CIMT values. Furthermore, the TSH levels were not correlated with fasting blood glucose, insulin or HOMA-IR, but there was a weak insignificant correlation between H-FABP and LDL-cholesterol (r=0.33, p=0.06). The serum fT4 levels were negatively correlated with total cholesterol, LDL-cholesterol and triglyceride levels (r=-0.689, p=0.00; r=-0.493, p=0.005; and r=-0.454, p=0.01, respectively), and the TSH levels were positively correlated with total cholesterol and LDL-cholesterol (r=0.485, p=0.06 and r=0.357, p=0.05 respectively).

Discussion

In the present study, we investigated whether serum H-FABP level increases in overt hypothyroidism. It is well known that overt hypothyroidism affects well known risk factors for cardiovascular diseases by increasing highly atherogenic LDL-cholesterol particles, the diastolic blood pressure, systemic vascular resistance, insulin resistance and C-reactive protein along with changes in coagulation. Some evidence also suggests that hypothyroidism may exacerbate the cardiovascular risks associated with smoking and insulin resistance (3). Additionally, younger male patients with subclinical hypothyroidism have increased levels of triglycerides and signs of low-grade inflammation. Subclinical hypothyroidism is also a predictor of cardiovascular disease in this patient group, with an odds ratio of 3.4 (95% confidence interval 1.6-6.8) for developing cardiovascular disease compared with euthyroid age-matched males (14). We also observed that the total cholesterol levels were the only variable that was significantly higher in the hypothyroid subjects when compared with the control subjects. In addition, we found higher CIMT values in the patient group than in the controls (0.53±0.08 mm vs. 0.48±0.05 mm, respectively) (p<0.05) but did not identify any other significant relationships between the CIMT values and other cardiovascular indicators, such as total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, insulin or HOMA-IR. Asik et al. (15) also found significantly elevated cardiovascular risk in patients with subclinical and overt hypothyroidism and that epicardial fat tissue was positively correlated with CIMT. Furthermore, Purohit (16) determined serum insulin levels, HOMA-IR, and BMI and found significantly higher levels in patients with overt hypothyroidism versus healthy controls. However, serum insulin levels, HOMA-IR, and BMI values were similar in the patient and control groups in our study.

Table 1. Demographic characteristics and biochemical data for the patients with hypothyroidism and the control subjects

	Patients (n=33)	Control (n=39)	p
Male/Female	2/31	5/34	0.326
*Age (years)	43.3±12.1	39.8±10.1	0.196
BMI (kg/m^2)	28.4±5.6	26.1±3.7	0.090
Current smokers, n (%)	6 (18.2)	0 (0)	<0.001
*Free T4 (ng/dL)	0.50±0.23	1.02±0.18	<0.001
*TSH (mIU/L)	70.40±55.94	2.10±1.52	<0.001
*HOMA-IR	2.28±1.65	2.01±0.92	0.404
*Total cholesterol (mg/dL)	220.00±73.67	191.00±35.73	0.030
*LDL cholesterol (mg/dL)	130.12±53.70	116.63±24.38	0.149
*HDL cholesterol (mg/dL)	49.23±11.99	51.68±13.79	0.746
*Triglycerides (mg/dL)	181.43±262.93	107.00±49.80	0.129
*H-FABP(pg/mL)	1515.87±2143.00	953.00±416.00	0.15
*CIMT (mm)	0.53±0.08	0.48±0.05	0.02

*Values are mean ± SD
SD: Standard deviation, BMI: Body mass index, TSH: Thyroid-stimulating hormone, HOMA-IR: Homeostasis model assessment of insulin resistance, LDL: Low-density lipoprotein, HDL: High-density lipoprotein, H-FABP: Heart-type fatty acid-binding protein, CIMT: Carotid intima-media thickness

Serum levels of H-FABP rapidly increase along with myocardium injury (9,17); therefore, H-FABP has recently been used as a diagnostic marker in conditions such as acute MI that cause myocardial damage. O'Donoghue et al. (18) also reported that high H-FABP levels were associated with an increased risk of adverse cardiovascular events and ACS, particularly death and heart failure risk in the first 10 months after the onset of this syndrome. Nakata et al. (19) concluded that H-FABP is a useful indicator for early detection of acute ischemic injury and a prognostic biochemical marker, particularly within the first six hours from the onset of chest symptoms in ACS. A study by Viswanathan et al. (20) also determined that higher H-FABP concentrations were associated with an increased risk of death and recurrent MI in patients with H-FABP levels of >6.48 µg/L and showed that this was independent of certain clinical risk factors, including troponin levels.

Previous studies have also demonstrated that H-FABP can be used as a marker for some conditions other than acute MI. Boscheri et al. (21) reported that H-FABP predicted mortality in patients with pulmonary embolism at intermediate risk and was significantly associated with impaired right ventricular function. Furthermore, they revealed that H-FABP had a better correlation with mortality than troponin I; hence, it might be used as a prognostic parameter to enable the optimization of a management strategy in the very difficult population of patients with pulmonary embolism at intermediate risk. Akbal et al. (22) demonstrated that serum H-FABP levels were significantly elevated in patients with metabolic syndrome associated with diabetes, and they found a significant positive correlation between H-FABP and waist circumference in those with metabolic syndrome. This suggests that it is a marker that offers promise for the detection of cardiac injury during the early asymptomatic period in these patients. Similarly, Karbek et al. (23) reported that H-FABP levels were significantly higher in patients with prediabetes (both impaired fasting glucose and impaired glucose tolerance) compared to control subjects and that H-FABP levels were positively correlated with CIMT. In contrast to these studies, Arslan et al. (24) found insignificant H-FABP levels in patients with prolactinoma. They evaluated preclinical atherosclerosis by measuring CIMT and demonstrated high values in comparison to healthy subjects.

Conclusion

In this study, there was no difference in H-FABP levels between patients with overt hypothyroidism and controls. However, the patients enrolled in our study were relatively young and had lower BMI values

compared with previous studies. In addition, most of our hypothyroid participants were female. These factors can affect the severity of cardiovascular risk and could have led to the insignificant H-FABP levels. Despite the high cardiovascular risk in overt hypothyroid patients, we determined that the H-FABP levels did not differ between patients and control subjects. Well-known cardiovascular risk markers and measuring CIMT rather than H-FABP might be employed to evaluate hypothyroid patients in terms of preclinical atherosclerosis.

Ethics

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: Esra Tutal, Mustafa Özbek, Design: Esra Tutal, Erman Çakal, Data Collection or Processing: Esra Tutal, Mustafa Özbek, Müyesser Saykı Arslan, Melia Karaköse, Aşkın Güngüneş, Taner Demirci, Mustafa Çalışkan, İlknur Öztürk Ünsal, Oya Topaloğlu, Gülfer Öztürk, Erman Çakal, Analysis or Interpretation: Esra Tutal, Müyesser Saykı Arslan, Erman Çakal, Literature Search: Esra Tutal, Writing: Esra Tutal. Conflict of Interest: No conflict of interest was declared by the authors. Financial Disclosure: The authors declared that this study received no financial support.

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