Plasma fetuin-A levels are reduced in patients with hypothyroidism

Okan Bakiner, Emre Bozkirli, Derun Ertugrul[1], Nurzen Sezgin[2] and **Eda Ertorer**

Department of Endocrinology and Metabolism Diseases, Faculty of Medicine, Baskent University, Dadaloglu Mah. Serinevler 2591 Sk., No:4/A 01250 Yuregir/ADANA, Adana, Turkey, [1]Department of Internal Medicine, Kecioren Teaching and Research Hospital, Ankara, Turkey and [2]Department of Biochemistry, Faculty of Medicine, Baskent University, Adana, Turkey

Correspondence should be addressed to O Bakiner
Email
okanbakiner@mynet.com

Abstract

Objective: To determine plasma fetuin-A levels in hypothyroid patients before and after treatment with L-thyroxine (T_4) and to determine the relation between plasma fetuin-A levels with cardiovascular risk factors.

Design: A prospective, controlled, single-blind study.

Methods: Forty-four treatment-naive female patients diagnosed with hypothyroidism and 39 age- and sex-matched control subjects were enrolled. Anthropometric measurements, blood pressure, plasma TSH, fetuin-A, free T_4, LDL-cholesterol, triglyceride, C-reactive protein, fibrinogen levels, and brachial artery flow-mediated dilatation were measured. All measurements were repeated after 3 months in the control group and 3 months after the attainment of euthyroidism with L-T_4 replacement in the hypothyroid group.

Baseline data were compared between the two groups. Posttreatment plasma fetuin-A levels of hypothyroid patients were compared with baseline levels of both groups. The relationship between plasma fetuin-A, TSH levels, and other cardiovascular risk factors was evaluated.

Results: Plasma fetuin-A levels were ~20% lower in hypothyroid female patients compared with the controls ($P=0.0001$). Fetuin-A levels increased by ~20% in hypothyroid patients after achievement of euthyroidism ($P=0.0001$) and were no longer different compared with controls ($P=0.38$). There was a negative correlation between plasma TSH and fetuin-A levels ($r=-0.79$; $P=0.001$). There was no significant correlation between plasma fetuin-A levels and cardiovascular risk factors within or between groups. The fetuin-A levels were normalized with thyroid hormone treatment.

Conclusion: Plasma fetuin-A levels are reduced in female patients with hypothyroidism, which are restored to normal during restoration of euthyroidism. There was no relation with cardiovascular risk factors.

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Introduction

Fetuin-A is a carrier plasma glycoprotein synthesized by the liver and has many functions (1). Its most famous function is on mineralization biology. Fetuin-A binds the calcium and phosphate in the medium to form calciprotein particle (CPP). CPP thus removes the calcium from the medium. It is internalized mainly by the Kupffer cells of the liver and macrophages in the splenic marginal zone, and carries out the calcium clearance (2, 3). Various studies have demonstrated an association between reduced plasma fetuin-A levels and increased vascular calcification and cardiovascular mortality in dialysis patients, increased risk of peripheral arterial disease, and coronary artery disease in patients with type 2 diabetes (4, 5, 6). Studies have therefore defined reduced plasma fetuin-A levels as a new cardiovascular risk factor and demonstrated that severity of atherosclerosis increases with low plasma fetuin-A levels (7, 8). In addition, in humans, fetuin-A has been suggested to provide an important link between obesity and insulin resistance (9, 10). It has been demonstrated to act as an endogenous

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ligand of Toll-like receptor 4 which is responsible for free fatty acid-induced inflammatory signaling in adipose tissue and insulin resistance (11). It has also been shown that in adipocytes, fatty acids induce fetuin-A gene and protein expression. Locally produced fetuin-A can act as a chemo-attractant for macrophages in adipose tissue, thus it may be regarded as responsible for lipid-induced inflammatory conditions (12). Hypothyroidism is associated with accelerated atherosclerosis and increased risk of coronary artery disease (13). Dyslipidemia, hypertension, endothelial dysfunction, hyperhomocysteinemia, elevated C-reactive protein (CRP) and plasminogen activator inhibitor-1 levels, and impaired endothelium-mediated vasodilatation are well known cardiovascular risk factors (14, 15, 16, 17, 18, 19). Coronary artery calcification is another factor increasing the cardiovascular risk (20), and coronary artery calcification has recently been shown to associate with reduced serum thyroxine (T_4) levels (21). Sato *et al*. (22) have demonstrated that triiodothyronine (T_3) increases matrix gla protein 3–8 times in vascular smooth muscle at physiological concentration. As matrix gla protein is a strong inhibitor of vascular calcification, this suggests that physiological levels of thyroid hormones may prevent vascular calcification and thus decrease cardiovascular risk. Although the anabolic effect of thyroid hormones on several proteins in the liver including fetuin-A has been demonstrated in *in vitro* experimental studies (23), it is not known whether clinically evident thyroid dysfunction is associated with the level of fetuin-A, which is another systemic calcification inhibitor involved in vascular calcification.

The objective of this study is to evaluate the impact of hypothyroidism on serum fetuin-A levels and to investigate the relationship between fetuin-A levels and conventional cardiovascular risk factors among women with hypothyroidism.

Subjects and methods

Patient selection

Newly diagnosed patients with hypothyroidism at the endocrinology outpatient clinic of Baskent University, Adana, Turkey between January 2012 and October 2012, were enrolled in the study. As serum fetuin-A levels and its metabolic effects may exhibit sexual dimorphism, only female patients were included (24, 25). All participants were between 18 and 50 years old and had hypothyroidism secondary to autoimmune thyroiditis with serum thyroid-stimulating hormone (TSH) levels above 10 mIU/l. The diagnosis of chronic autoimmune thyroiditis was established in the presence of elevated levels in at least one of antithyroglobulin (ATA) and antithyroid peroxidase (anti-TPO) antibody, and/or observation of ultrasonographic changes in thyroiditis. Exclusion criteria were: postmenopausal, smoking, or pregnant patients; patients with history of L-T_4 replacement therapy; calcium metabolism disorders, or receiving any medications with potential effects on calcium metabolism including loop diuretics or thiazides; and patients with concomitant metabolic diseases, coronary artery disease, or other disorders that might alter protein or calcium metabolism including chronic renal failure, liver failure, malnutrition, or malignancy. An age-sex-matched healthy group of volunteers were included as controls. This study was approved by Baskent University institutional review board and ethics committee (Project no: KA 12/277). The written informed consent from all participants was obtained at inclusion.

Patients who failed to attend the follow-up visits regularly or attain target TSH levels (0.45–4.12 mIU/l) with L-T_4 therapy (26), received any medications with potential effects on calcium metabolism during the follow-up, or became pregnant were removed from the study.

Method

This is a prospective, controlled, single-blind study. Anthropometric measurements were carried out and BMI was calculated in all participants. The average of three consecutive resting arterial blood pressure measurements was recorded. Venous blood samples were obtained from the forearm to measure plasma TSH, free T_4 (fT_4), LDL-cholesterol, HDL-cholesterol, triglyceride, high-sensitivity CRP (hs-CRP), and fibrinogen levels after an overnight fasting. The blood samples obtained for fetuin-A measurement were stored at −80 °C. Flow-mediated dilatation (FMD) measurement was carried out at the brachial artery for each participant. These measurements and analyses were repeated in female patients with hypothyroidism following the treatment (at 3 months of the attainment of target plasma TSH levels with L-T_4 replacement therapy). Similarly, the measurements were repeated in the control group after 3 months from the baseline. Baseline data were compared between the two groups. Baseline data were also compared with the posttreatment data in the patient group. Posttreatment data of the patient group was compared with controls. The relationship between plasma fetuin-A levels and plasma TSH levels and other cardiovascular risk factors was evaluated.

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Analysis

Serum TSH levels were measured with the chemiluminescence method using the Abbott-Architect analyzer, and ATA and anti-TPO levels were measured with the electrochemiluminescence immunoassay method using the Modular E170 analyzer (Roche Diagnostic).

Serum fibrinogen was measured with the Clauss coagulation method using the BCT auto-analyzer (Siemens Healthcare Diagnostics, Newark, DE, USA). Serum total cholesterol, HDL-cholesterol, and triglyceride levels were measured with the original kits of Roche Modular auto-analyzer (Roche Diagnostic GmbH). LDL-cholesterol level was calculated with the Friedewald formula (27).

hs-CRP level was measured with the particle-enhanced immunonephelometric method using the BN II System (Siemens Healthcare Diagnostics, Marburg, Germany).

Plasma fetuin-A level was measured with the human fetuin-A ELISA kit provided by Biovendor Laboratory Medicine (Brno, Czech Republic). The minimum assay sensitivity of human fetuin-A ELISA kit was 0.35 ng/ml, and the inter/intra-measurement variability coefficient was determined as $<6.5\%$.

FMD was measured ultrasonographically at a room with standard heat-light features at 22–25 °C. All measurements were carried out at supine position, maintaining the probe parallel to the long axis of the right brachial artery at 2–5 cm above the antecubital fossa, and brachial artery diameters were obtained at the first time during resting. The measurement was standardized to measure the diameter between the vessel wall closest to the probe and the imaginary line between media and adventitia of the wall far from the lumen. All measurements were carried out at B-mode gray scale. The sphygmomanometer on the forearm was expanded (ca. 250 mmHg pressure) immediately afterwards, the pressure was maintained for 5 min, and the measurements were repeated (at approximately the same place) one min after the reduction of pressure on the sphygmomanometer. FMD was calculated by the FMD = variance in arterial diameter/basal arterial diameter (%) formula. The Antares US system (Siemens, Inc., Mountain, View, CA, USA), multifrequency (4–9 MHz) linear probe was used in this performance.

Statistical analysis

The Social Package for Statistical Sciences (SPSS) version 18.0 was used in the statistical analyses of the data. Categorical data were summarized as number and percentages, and numerical data were summarized as mean and SD. (median and minimum–maximum when necessary). Kolmogorov–Smirnov test was used to test whether numerical data conformed to normal distribution. Intergroup comparison of numerical data was carried out with independent groups *T*-test when the assumptions were met, and with the Mann–Whitney *U* test when assumptions were not met. In the comparison of before–after-dependent numerical measurements, dependent groups *T*-test was used in dependent groups meeting the assumptions, and with the Wilcoxon Signed-rank test was used when assumptions were not met. The correlation between fetuin-A and FMD was analyzed with the Pearson correlation coefficient. A P value of 0.05 was used to determine statistical significance in all tests.

Results

Forty-four female patients with hypothyroidism meeting the study inclusion criteria were included in the patient group. Thirty-nine healthy volunteers were included in the control group. Five patients in the hypothyroidism group were removed from the study due to failure in reaching target plasma TSH values in follow-up and attending control visits, and four subjects in the control group were removed for failure in obtaining control data at 3 months. As a result, the study population consisted of 39 female patients and 35 controls.

Baseline data of both groups and their statistical comparisons are presented in Table 1.

Plasma fetuin-A levels were significantly lower in female patients with hypothyroidism compared with the controls at baseline ($P=0.0001$). A significant negative correlation was determined between plasma TSH and plasma fetuin-A levels in all subjects at baseline ($P=0.001$, $r=-0.79$). This negative correlation persisted in hypothyroid patients as well ($P=0.01$, $r=-0.61$). Positive correlation was found between serum fT_4 and plasma fetuin-A levels in patients at inclusion ($P=0.021$, $r=0.49$). None of the baseline cardiovascular risk factors exhibited correlation with plasma fetuin-A levels in the patient group (Table 2). Final median serum TSH level was 2.2 (0.5–4.1) mIU/l and was lower than baseline values ($P=0.0001$) in the patient group at the end of the study period. Mean L-T_4 dose given to normalize TSH was calculated as 1.49 ± 0.71 μg/kg per day per patient. Final serum TSH levels of the patient group were statistically indifferent to that of the control group ($P=0.17$). Baseline and 3-month serum TSH levels of the control group were not different either ($P=0.36$). Median plasma fetuin-A

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Table 1 Baseline characteristics and statistical comparisons of the two groups.

	Patient ($n=39$)	**Control** ($n=35$)	
	Median (Min–Max)	Median (Min–Max)	***P***
Patient age (years)	32.0 (21.0–55.0)	27.0 (18.0–46.0)	**0.101**
BMI (kg/m^2)	27.0 (19.0–38.0)	24.1 (15.0–37.7)	**0.009**
Fetuin-A (ng/ml)	303.3 (230.1–379.5)	387.4 (298.8–422.9)	**0.0001**
TSH (mIU/l)	18.9 (10.4–100.0)	1.3 (0.4–3.7)	**0.0001**
Systolic BP (mmHg)	110.0 (100.0–140.0)	110.0 (100.0–120.0)	0.106
Diastolic BP (mmHg)	70.0 (60.0–100.0)	60.0 (50.0–80.0)	**0.012**
Antithyroglobulin Ab (IU/ml)	82.4 (10.0–1246.0)	10.3 (10.0–120.7)	**0.001**
Antimicrosomal Ab (IU/ml)	310.4 (5.0–1525.0)	5.3 (2.8–29.0)	**0.0001**
Free T_4 (mol/l)	8.2 (5.0–15.1)	14.5 (9.4–15.9)	**0.001**
Total cholesterol (mg/dl)	192.5 (112.0–320.0)	150.0 (112.0–218.0)	**0.007**
LDL (mg/dl)	113.0 (59.0–214.0)	90.0 (50.8–143.0)	**0.025**
HDL (mg/dl)	49.3 (23.0–83.9)	50.0 (30.0–70.7)	0.773
Triglyceride (mg/dl)	108.5 (41.0–761.0)	68.0 (34.0–123.0)	**0.020**
CRP (mg/l)	3.0 (2.6–41.8)	3.0 (0.4–14.0)	0.093
Fibrinogen (g/l)	3.2 (1.9–5.7)	2.5 (2.0–5.4)	0.063
FMD (%)	5.0 (2.3–10.5)	6.5 (2.0–10.2)	**0.042**

TSH, thyroid-stimulating hormone; BP, blood pressure; C, cholesterol; CRP, C-reactive protein; FMD, flow-mediated arterial dilatation. Statistically significant *P* values are expressed as bold.

level was 362.5 (282.7–454.1) ng/ml following L-T_4 treatment and was significantly higher than the baseline values ($P=0.0001$) in the patient group. There was no significant difference between the plasma fetuin-A levels after achievement of euthyroidism in the patient group and the baseline plasma fetuin-A levels of the control group ($P=0.388$). In addition, baseline and 3-month plasma fetuin-A levels of the control group were similar ($P=0.286$). There was no correlation between post-treatment TSH levels and posttreatment plasma fetuin-A levels ($P=0.054$, $r=-0.12$).

Changes in cardiovascular risk factors following TSH normalization are demonstrated in Table 3 in the study group. BMI, diastolic blood pressure, total cholesterol, and LDL-cholesterol levels were lower and flow-mediated arterial dilatation was higher in the patient group at the end of study period (Table 3). None of them were correlated with plasma fetuin-A changes. Comparison of posttreatment cardiovascular risk factors in patients and baseline cardiovascular risk factors in controls are presented in Table 4.

A regression analysis was carried out to determine the impact of cardiovascular risk factors on plasma fetuin-A levels independently from hypothyroidism. The dependent variable was plasma fetuin-A level. No significant relationship was determined between fetuin-A levels and any of the cardiovascular risk factors. However, hypothyroidism and TSH levels were found to be the two parameters affecting fetuin-A levels in all subjects ($P=0.024$ and $P=0.021$ respectively).

Discussion

In this study, we found that hypothyroidism was associated with lower plasma fetuin-A levels, independent of other cardiovascular risk factors. Moreover, fetuin-A levels in the patient group with L-T_4 replacement were returned to the normal levels as in controls. This is the first study demonstrating the relation between low fetuin-A levels and hypothyroidism, which are both cardiovascular risk factors (5, 13).

Table 2 Correlation between plasma fetuin-A levels and baseline cardiovascular risk factors in patients with hypothyroidism.

Baseline cardiovascular risk factors	***r***	***P***
TSH	**−0.61**	**0.01**
BMI	0.67	0.06
Systolic BP	−0.134	0.488
Diastolic BP	0.073	0.707
Total C	0.156	0.436
LDL	0.166	0.407
HDL	−0.092	0.648
Triglyceride	0.393	0.143
CRP	0.115	0.562
Fibrinogen	0.332	0.084
Flow-mediated arterial dilatation	0.141	0.493

TSH, thyroid-stimulating hormone; BP, blood pressure; C, cholesterol; CRP, C-reactive protein. Statistically significant *P* values are expressed as bold.

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Table 3 Alteration in cardiovascular risk factors following treatment in patients with hypothyroidism.

	Baseline	**Posttreatment**	
	Median (Min–Max)	Median (Min–Max)	***P***
BMI (kg/m^2)	27.0 (19.0–38.0)	23 (15.5–34.0)	**0.009**
Total cholesterol (mg/dl)	192.5 (112.0–320.0)	180.0 (113.0–286.0)	**0.005**
LDL (mg/dl)	113.0 (59.0–214.0)	101.0 (66.8–207.0)	**0.048**
HDL (mg/dl)	49.3 (23.0–83.9)	44.0 (28.3–69.0)	0.052
Triglyceride (mg/dl)	108.5 (41.0–761.0)	104.0 (41.0–481.0)	0.954
Systolic BP (mmHg)	110.0 (100.0–140.0)	110.0 (100.0–145.0)	0.106
Diastolic BP (mmHg)	70.0 (60.0–100.0)	65.0 (60.0–85.0)	**0.012**
CRP (mg/l)	3.0 (2.6–41.8)	3.0 (3.0–51.4)	0.615
Fibrinogen (g/l)	3.2 (1.9–5.7)	2.8 (2.0–5.3)	0.298
FMD (%)	5.0 (2.3–10.5)	7.5 (3.0–15.2)	**0.0001**

BP, blood pressure; CRP, C-reactive protein; FMD, flow-mediated arterial dilatation. Statistically significant *P* values are expressed as bold.

Fetuin-A is a glycoprotein synthesized mainly in the liver. A major site of plasma protein production is the hepatocyte, and various *in vitro* techniques have been used to modulate the production of these essential proteins. Gromakova *et al*. (28) have reported that thyroid status affects the protein synthesis in liver by altering the RNA polymerase activity, and that protein synthesis is increased in hyperthyroidism and decreased in hypothyroidism. Lin *et al*. (23) carried out an *in vitro* experimental study and demonstrated that protein synthesis is increased in direct interaction with the promoter region of major proteins synthesized by the liver including fetuin-A (α-2 hs glycoprotein) via the α-1 thyroid receptors with effects of T_3. The results of these studies may explain the low fetuin-A levels in patients with hypothyroidism and its normalization via maintaining euthyroidism. In a recent study investigating plasma fetuin-A levels among thyroidectomized and radioactive iodine-ablated patients, Gagnon *et al*. (29) reported insignificant fetuin-A change with recombinant human TSH. This finding supports that fetuin-A synthesis affected thyroid hormone status more than serum TSH levels. In another recent clinical study, Pamuk *et al*. (30) demonstrated high fetuin-A levels in patients with hyperthyroidism, which have returned to normal with achieving euthyroidism. The latter study is also a supportive mirror image of our data. Lack of a clinical study investigating plasma fetuin-A levels in patients with hypothyroidism makes our study unique in medical literature, and is also a limitation for making a comparison with other human studies. However, the significant negative correlation determined between plasma TSH and fetuin-A levels in all subjects ($P=0.001$, $r=-0.79$) was in conformity with the *in vitro* experimental studies (23, 28).

Table 4 Comparison of cardiovascular risk factors following treatment in patients with hypothyroidism and controls.

	Patients (measurements at month 3)	**Control** (measurements at baseline)	
	Median (Min–Max)	Median (Min–Max)	***P***
TSH (mIU/l)	2.2 (0.3–4.4)	1.3 (0.4–3.7)	0.171
BMI (kg/m^2)	23.0 (15.5–34.0)	24.1 (15.0–35.7)	0.263
Total cholesterol (mg/dl)	180.0 (113.0–286.0)	150.0 (112.0–218.0)	**0.035**
LDL (mg/dl)	101.0 (66.8–207.0)	90.0 (50.8–143.0)	0.068
HDL (mg/dl)	44.0 (28.3–69.0)	50.0 (30.0–70.7)	0.339
Triglyceride (mg/dl)	104.0 (41.0–481.0)	68.0 (34.0–123.0)	**0.018**
Systolic BP (mmHg)	110.0 (100.0–145.0)	110.0 (100.0–120.0)	0.635
Diastolic BP (mmHg)	65.0 (60.0–85.0)	60.0 (50.0–80.0)	0.324
CRP (mg/l)	3.0 (3.0–51.4)	3.0 (0.4–14.0)	**0.040**
Fibrinogen (g/l)	2.8 (2.0–5.3)	2.5 (2.0–5.4)	0.094
FMD (%)	7.5 (3.0–15.2)	6.5 (2.0–10.2)	0.988

TSH, thyroid-stimulating hormone; BP, blood pressure; CRP, C-reactive protein; FMD, flow-mediated arterial dilatation. Statistically significant *P* values are expressed as bold.

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There is a strong association between coronary artery calcification and cardiovascular events (20). Dystrophic intimal calcification in atherosclerotic plaque allows progression of atherosclerosis (31). Experimental studies have demonstrated that calcium deposition in matrix vesicles of vascular smooth muscle cells (VSMC) and pericytes results in apoptosis and macrophage activation with the help of release of inflammatory mediators (32, 33). This in turn allows easier progression of the atherosclerotic plaque and rupture with impairment of biomechanical stability (34). A cell culture study carried out with VSMC has demonstrated that fetuin-A decreases intracellular vesicular calcium deposition and thus prevents VSMC calcification and apoptosis (35). Increased vascular calcification has been demonstrated in hypothyroidism, which might result from decreased levels of matrix gla protein, a systemic calcification inhibitor (21, 22). However, the relationship between hypothyroidism and fetuin-A, another systemic calcification inhibitor, has been demonstrated in our study and might be yet another cause of vascular calcification in hypothyroidism. Further *in vitro* studies should be carried out to elucidate this issue.

Macrophages migrating into the lesion in the early stage of atherosclerosis engulf oxidized LDL particles to form foam cells and secrete chemokines. So the inflammatory process goes on and results in maturation of atherosclerotic plaque (36). Oxidized LDL enters the macrophage via scavenger receptors (37). CPPs synthesized in the presence of fetuin-A has been suggested to play a protective role against the inflammatory component of atherosclerosis by decreasing oxidized-LDL levels inside the macrophage through competition in entering the cell, by binding the scavenger receptors on macrophage surface in the atheroma plaque (3, 38). These data suggest that demonstration of reduced fetuin-A levels in patients with hypothyroidism might be deemed as the establishment of a novel cardiovascular risk factor. Studies have demonstrated a positive correlation between plasma fetuin-A levels and other cardiovascular risk factors including high arterial blood pressure, central obesity, high triglyceride, and LDL-cholesterol levels, and impaired FMD indicating vascular resistance (39). Fetuin-A is an endogenous inhibitor of the insulin receptor tyrosine kinase and fetuin-A knockout mice exhibit an increased insulin sensitivity (40, 41). Recent studies have proposed association between high fetuin-A levels and cardiovascular risk factors, such as increased risk of type 2 diabetes, insulin resistance, hepatosteatosis, and metabolic syndrome (10, 42, 43). Although some of these cardiovascular risk factors were present in our patient group, fetuin-A level was found to be lower independent of those mentioned earlier, suggesting a predominant effect of hypothyroidism on fetuin-A levels. Improvement in some cardiovascular risk factors, (i.e. serum lipids, BMI, blood pressure, and FMD) was observed in our patient group at the end of the study period, with maintained euthyroidism. These changes did not correlate with plasma fetuin-A changes, which suggested that hypothyroidism exerted an independent effect on plasma fetuin-A levels. However, the exact relationship between reduced fetuin-A levels and increased cardiovascular risk in patients with hypothyroidism cannot be determined due to the lack of a detailed cardiovascular examination in the patient group and follow-up of long-term cardiovascular events. We can only make a projection with our results in conjunction with the experimental and clinical data of previous studies.

Another major point to mention is whether our methodology of fetuin-A measurement was valid for patients with hypothyroidism. Although a recent study has demonstrated that a sub-picogram-sensitive rapid chemiluminescent immunoassay method is 125 times more sensitive than the classical commercial ELISA method, ELISA is still the gold standard and extensively used method in human fetuin-A screening (44). In our study, we also used the commercially available ELISA method to measure fetuin-A levels. Comparable mean fetuin-A level with that of healthy controls of our study and of another study (45), reduction in fetuin-A level to near-normal in patients with hypothyroidism following treatment and lack of change in fetuin-A levels of the control group during follow-up were supportive of the reliability of our study.

Consequently, reduced fetuin-A level, deemed as a cardiovascular risk factor, was determined in our female patients with hypothyroidism. Reduced fetuin-A level is considered to aggravate cardiovascular events via vascular calcification and inflammatory process in atherosclerosis in these patients. Currently, it is not known whether reduced plasma fetuin-A level is a marker of cardiovascular risk in patients with hypothyroidism. However, further experimental and observational studies might elucidate this. Confirmation of the role of reduced fetuin-A level as a cardiovascular risk marker may allow its use in initiating treatment in special patient and disease groups including cardiovascular disease concomitantly with hypothyroidism, advanced age, or subclinical hypothyroidism.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

O Bakiner conceived and designed the study, and drafted the manuscript. E Bozkirli helped to draft the manuscript, helped in acquisition and interpretation of data, and also edited the language. D Ertugrul helped to draft the manuscript, helped in acquisition and interpretation of data, and also performed the statistical analysis. N Sezgin carried out the fetuin-A assays. E Ertorer helped to draft the manuscript, helped in the design of the study, was involved in interpretation of data and in revising the manuscript for important intellectual content. All authors read and approved the final version of the manuscript.

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