IS SALUSIN-ALPHA A NEW MARKER OF CARDIOVASCULAR DISEASE RISK IN HYPOTHYROIDISM?

H. Usta Atmaca^{1,*}, F. Akbas¹

¹*Istanbul Training and Research Hospital, Dept. of Internal Medicine, Samatya, Istanbul, Turkey*

Abstract

Introduction. Salusins are multifunctional endogenous peptides shown in human and rat tissues. Serum salusin α level is decreased in coronary artery disease and lack of salusin α enhances coronary atherosclerosis. Hypothyroidism is a chronic inflammatory disease that has a high risk of developing cardiovascular disease. Here we aimed to search the relationship of overt hypothyroidism and subclinical hypothyroidism with salusin α and other inflammatory markers, also the effect of L-thyroxine treatment on these findings.

Material and Methods. 32 patients with overt hypothyroidism taking L-thyroxine treatment, 18 patients with subclinical hypothyroidism without treatment and 25 healthy patients as control group were included in the study. Serum salusin α , TNF α , sCRP, glucose, insulin and lipid levels were tested for all groups and results were evaluated with SPSS statistical analysis method.

Results. HDL, sCRP, salusin mean values were statistically significantly different in all 3 groups. HDL level was statistically significantly higher in control group compared to treatment group. sCRP level was higher and salusin level was lower in both treatment and non-treatment hypothyroidism groups compared to control group. When treatment and non-treatment hypothyroidism groups were compared, there was no statistically significant difference for salusin α , but HDL level was high and insulin level was low statistically significant in treatment group.

Conclusions. Salusin α that is shown to be protective for coronary artery disease and hypertension, is found to be significantly low in hypothyroidism, thus it is a marker that increases the cardiovascular disease risk in this specific patient group.

Key words: Salusins, hypothyroidism, cardiometabolic risk, inflammation.

INTRODUCTION

Salusins (salusin-alpha and salusin-beta) are multifunctional endogenous vasoactive peptides consisting of 20 and 28 aminoacids that were

determined in human and rat tissues. They were first defined by Shichiri *et al.* (1) in 2003 and their secretion from the cardiovascular system, central nervous system and kidneys (2) as well as their presence in human plasma and urine were shown (2).

Salusins are expressed in high amounts in vascular smooth muscle cells and endothelial cells as preprosalusin (1). Salusin β contains higher amounts of hydrophobic aminoacids compared with salusin alpha and biochemical features of each are different. The secretion of salusin beta is induced by inflammatory cytokines such as tumor necrosis factor-alpha (TNF-alpha) (3, 4).

Salusin alpha has hemodynamic effects majorly on the cardiovascular system such as lowering blood pressure and slowing the heart rate. The effects of salusins on growth promotion, induction of hypertrophy and inhibition of apoptosis on rat cardiomyocytes were reported (5, 6). After bolus injection of salusin beta, transient and rapid hypotension with bradycardia through cholinergic mechanisms and direct negative inotropic effects have been demonstrated whereas its direct vasodilator effects on vessels could not be shown (7). As salusin beta has atherogenic effects, conversely salusin alpha is considered anti-atherogenic. This difference had been related to their opposite effects on acyl-coenzyme A cholesterol acyl transferase-1 (ACAT-1) enzyme. Reversal effect of salusin alpha on atherosclerosis depends on the inhibition of ACAT-1 expression and on the decrease of acyl-coenzyme A levels, by diminishing foam cell formation on human monocyte derived macrophages (3). In recent studies, this marker was found to be protective against cardiovascular diseases and its levels were found to decrease in chronic inflammatory diseases, systemic lupus erythematosus and systemic sclerosis (8), and in chronic renal failure (9); whereas its levels were found to increase in Behcet's disease and rheumatoid arthritis (10).

*Correspondence to: Hanife Usta Atmaca MD, Istanbul Training and Research Hospital, Dept. of Internal Medicine, Samatya, Istanbul, 34000, Turkey, E-mail: hanifeusta@yahoo.com

Hypothyroidism is a chronic inflammatory disease that predisposes accelerated atherosclerosis and hypertension development. These effects are associated with its negative effects on lipid profile, increase in lipoprotein a levels (11), and increase in serum homocysteine and c-reactive protein (CRP) levels, as well as escalation in endothelial dysfunction and arterial stiffness (12). Hypothyroidism causes atherosclerotic cardiovascular disease development also by negative chronotropic and inotropic effects reducing oxygen demand of the myocardium, hence provoking an underlying coronary ischemia (13, 14).

Although the exact cause of hypertension development in hypothyroidism is not fully understood, structural changes in vascular tissue due to thyroid hormone deficiency and to the presence of local factors altering the total peripheral resistance may be considered as the mechanism of action (15). In addition, it was shown that changes in autonomic nervous system result in hemodynamic alterations and an increase in plasma norepinephrine levels. So, thyroid hormone replacement therapy was shown to decrease central dopaminergic activity and to lower blood pressure (16).

Subclinical hypothyroidism is a chronic inflammatory disease commonly reported especially in middle or advanced aged women that is characterized by increased serum thyroid stimulating hormone (TSH) levels but normal thyroxine (fT4) and normal triiodothyronine (fT3) levels. Its prevalence ranges between 10-15% (17-19). Although the upper limit of TSH remains controversial, values above 2.5 mU/L are considered as early or mild thyroid gland dysfunction (20).

The aim of this study was to determine: serum salusin alpha levels in patients with overt hypothyroidism under L-tyroxine treatment, serum salusin alpha levels in untreated patients with subclinical hypothyroidism, to define its association with classical cardiovascular risk factors (body mass index, glucose, HDL-cholesterol and LDL-cholesterol, insulin, s C-reactive protein and TNF- alpha); hence to investigate its role as an early clinical cardiac marker in hypothyroidism and to investigate the effect in L-tyroxine treatment on cardio-metabolic indicators and salusin alpha levels.

MATERIAL AND METHODS

The study included 32 patients (31 women, 1 man) with overt hypothyroidism under L-tyroxin treatment (group A), 18 women with subclinical

hypothyroidism without medication (group B), and 25 healthy people (21 women, 4 men) as the control group (group C). The patients were informed about the study procedures and gave their written informed consent. The institutional ethics committee approved the study protocol. All study procedures were in agreement with Helsinki declaration.

All patients with hypothyroidism had the diagnosis of autoimmune hypothyroidism with thyroid antibody positivity (anti-TPO levels ≥ 400 IU/L). The exclusion criteria were; morbid obesity (BMI > 40 kg/m²), smoking, alcohol consumption history, taking any other type of medications, presence of familial or secondary dyslipidemia, or presence of systemic diseases.

In all cases, carotid intima media thickness (IMT) was evaluated by carotid Doppler ultrasound and cases with normal IMT were included in the study while patients with overt atherosclerosis were excluded.

In patients with subclinical hypothyroidism, other than the above criteria, patients with high thyroid antibody levels with serum TSH levels of within range 2.5 – 6 mU/L with normal free T4 levels and among patients with overt hypothyroidism, cases with normal TSH levels (between 2.5-5 mU/L) with treatment were included.

In all cases, body mass index (BMI) was calculated [BMI=weight (kg)/height (m²)] and recorded. Before study, all patients were informed about tests and their clinical explanations and their written informed consents were obtained. After twelve hours of night fasting, the blood samples were taken from ante-cubital veins in the morning around 08:00-10:00 a.m. after 20 minutes of resting in sitting position. 10 ml of blood samples were collected on red-capped, vacuumed tubes containing gel separators without any additives (Becton Dickinson Diagnostics, Franklin Lakes, USA). After waiting 30 minutes for blood to clot, test tubes were centrifuged at 3000 g for 10 minutes and serum was separated. Serum samples were divided into two portions without waiting and stored at -80 °C until the study day. On the night before study, serum samples were warmed up to +4 °C and were kept at room temperature in the laboratory until the morning of study. Glucose, total cholesterol, LDL cholesterol, HDL cholesterol, triglyceride levels were studied with Beckman Coulter AU 2700 plus (Beckman Coulter, Inc., Fullerton, USA) auto analyzer with spectrophotometric method in one run. The daily internal quality control results for those parameters

were in ± 1 SD limits. Insulin, TSH, T3, fT4, anti-thyroglobulin (anti-Tg) and anti-thyroid-peroxidase (anti-TPO) levels were obtained with Beckman Coulter Unicel DXI 800 machine (Beckman Coulter, Inc., Fullerton, USA) with chemiluminescence-immunoassay method. The daily internal quality control results for those parameters were in ± 1 SD limits. HOMA-IR (Homeostatic Model Assessment-Insulin Resistance) scores were calculated with the formula of $\text{HOMA-IR} = \text{fasting blood glucose (mg/dL)} \times \text{fasting blood insulin (mIU/mL)} / 405$. High sensitive C-reactive protein (hsCRP) was studied with Beckman Coulter Image 800 machine (Beckman Coulter, Inc., Fullerton, USA) with nephelometric method. Human TNF-alpha (Assaypro, St. Charles, USA) and human salusin alpha (Eastbiopharm Co., Ltd., Chinese) levels were demonstrated by their separate and specific ELISA kits.

Statistical analysis was performed using SPSS 15.0 for Windows program. Descriptive statistics were reported as number and percentage for categorical variables and as mean and standard deviation for numerical variables. When the numerical variables were distributed normally, in comparison with more than 2 independent groups One-Way Anova, if those groups were not distributed normally, Kruskal-Wallis tests were performed. In non-parametric tests, subgroup analyses were performed with Mann Whitney U test and commented with Bonferroni correction. The ratios of categorical variables between groups were tested

with chi square test. When parametric test criteria could not be achieved, the associations of numerical variables were investigated with Spearman correlation analysis. The discriminating factors were tested with linear regression analysis. The statistical alpha significance level was regarded as $p < 0.05$.

RESULTS

The study included 32 patients (31 women, 1 man) with overt hypothyroidism under L-thyroxin treatment with a mean age of 40.3 ± 12.2 years, 18 untreated women with subclinical hypothyroidism with a mean age of 38.4 ± 9.2 years, and 25 healthy subjects (21 women, 4 men) as control group with a mean age of 37.3 ± 9.9 years. The demographic, clinical and laboratory parameters of all participants are summarized in Table 1.

Among those parameters, the mean HDL, hsCRP, and salusin alpha levels were statistically significantly different between the groups ($p = 0.018$, $p < 0.001$ and $p = 0.003$, respectively). HDL cholesterol was higher in control group compared with the treatment group ($p = 0.018$). hsCRP levels were statistically significantly higher in both subclinical and overt hypothyroidism groups compared with control cases ($p < 0.001$ and $p = 0.002$, respectively). It was found that salusin alpha levels were statistically significantly lower in both hypothyroidism groups than in the control group ($p = 0.003$ and $p = 0.016$ respectively).

Table 1. The distribution of groups regarding demographic, clinical and laboratory parameters and subgroup analysis

		Control N (%)	Treated	Not Treated	p
Gender	Female	21 (84.0)	31 (96.9)	18 (100)	0.066
	Male	4 (16.0)	1 (3.1)	0 (0.0)	
		Mean. $\pm$ SD	Mean. $\pm$ SD	Mean. $\pm$ SD	p
Age		37.3 ± 9.9	40.3 ± 12.2	38.4 ± 9.2	0.558
BMI		25.0 ± 3.6	26.6 ± 4.2	25.1 ± 6.1	0.179
Glucose		78.6 ± 12.3	81.0 ± 13.0	86.3 ± 17.4	0.426
Total cholesterol		185.1 ± 38.3	-	168.9 ± 50.4	0.257
Triglyceride		106.0 ± 133.7	159.5 ± 439.0	83.8 ± 46.9	0.520
HDL cholesterol		46.8 ± 11.4	40.0 ± 13.2	45.5 ± 16.6	0.018
LDL cholesterol		121.6 ± 30.4	126.7 ± 36.6	118.8 ± 36.5	0.720
HsCRP		0.7 ± 1.6	3.3 ± 4.0	1.4 ± 1.2	< 0.001
Insulin		6.9 ± 7.2	5.7 ± 3.4	5.4 ± 3.9	0.569
Salusin-alpha		740.7 ± 418.6	486.5 ± 327.0	480.2 ± 366.9	0.003
TNF		0.17 ± 0.13	0.18 ± 0.18	0.13 ± 0.03	0.705

Subgroup Analysis

P	Control vs. Treated	Control vs. Not Treated	Treated vs. Not Treated
HDL	0.018	0.588	0.195
HsCRP	< 0.001	0.002	0.110
Salusin- α	0.003	0.016	0.635

Table 2. The correlation between salusin alpha and hsCRP levels with other parameters

	sCRP		Salusin- α	
	rho	p	rho	p
Salusin-α	-0.095	0.521		
Age	0.452	0.001	0.030	0.838
BMI	0.269	0.070	-0.092	0.535
Glucose	0.319	0.027	-0.080	0.579
Total cholesterol	0.292	0.239	-0.026	0.919
Triglyceride	0.426	0.003	0.037	0.797
HDL-cholesterol	0.074	0.618	0.016	0.910
LDL-cholesterol	0.507	<0.001	0.013	0.928
Insulin	0.174	0.237	0.314	0.028
TNF-α	-0.247	0.090	-0.115	0.428

Table 3. Relationship of salusin with other parameters

	B	Beta*	p
Constant	891.9		
Age	3.287	0.106	0.602
BMI	-11.403	-0.162	0.358
Glucose	-1.815	-0.078	0.741
Triglyceride	-0.059	-0.062	0.735
LDL cholesterol	-1.245	-0.131	0.550
HDL cholesterol	-0.704	-0.030	0.890
Insulin	19.000	0.197	0.253
TNF-α	-6.428	-0.003	0.986

*Beta: regression coefficient

Figure 1. Salusin mean values.

There was not a statistically significant difference regarding salusin alpha levels between patients who are under L-thyroxin treatment and those who are not (Tables 2, 3 and Fig. 1).

In thyroiditis patient group, hsCRP levels were positively correlated with age, triglyceride and with LDL levels ($p=0.001$, $p=0.003$ and $p<0.001$, respectively), whereas salusin levels were only positively correlated with insulin in a statistically significant way ($p=0.028$). There has not been found any correlation determined between salusin and hsCRP levels ($p=0.521$).

DISCUSSION

Some plasma inflammatory biomarkers are associated with cardiovascular disease risk and these biomarkers play an important role in prediction of disease progression and presence of premature atherosclerosis (21). In this study, we have found that, a new biomarker, salusin alpha decreases in hypothyroidism which is a low grade inflammatory disease suggesting that in hypothyroidism, among factors causing premature atherosclerosis and hypertension development, decreased salusin alpha levels may also play a role.

In our study, salusin alpha levels were statistically significantly lower in the control group compared to patients with the diagnosis of hypothyroidism with or without l-thyroxine treatment. There was not a statistically significant difference regarding salusin alpha levels between patients who are under L-thyroxin treatment and those who are not. It is clearly known that L-thyroxin treatment improves the cardiovascular disease risk factors in hypothyroidism (22). However, in our study, salusin alpha levels did not indicate this correlation.

A limited number of studies related to the decrease of salusin alpha levels in other systemic

chronic diseases such as rheumatologic diseases included systemic lupus, systemic sclerosis (3); chronic renal failure (10), and hypertension (23) suggested that decreased salusin alpha levels may be an early marker of silent cardiovascular disease.

In the study of Watanabe *et al.* (23) in 70 patients with hypertension, serum salusin alpha levels were found to be decreased compared to control cases. In the same patient group, increased intima media thickness and plaque score were obtained and a negative correlation between serum salusin alpha levels and carotid intima media thickness was reported (23). In another study, in patients with coronary artery disease, decreased serum salusin alpha levels was found and among those patients an independent and negative correlation was reported between salusin alpha levels and coronary artery disease severity defined with coronary angiography (24).

Other factors strengthening and promoting the association of hypothyroidism with early atherosclerosis and other cardiovascular diseases are related with atherogenic lipid profile, diastolic hypertension, and endothelial dysfunction. Hyperlipidemia reduces endothelial nitric oxide synthesis and increases asymmetric dimethyl arginine levels promoting endothelial dysfunction (25, 26). In a study of Türemen *et al.* endothelial dysfunction was demonstrated in patients with hypothyroidism, (27). In other studies, hyperhomocysteinemia and increased CRP levels which are both atherogenic were also found to be involved in endothelial dysfunction (28).

The effects of hypothyroidism on insulin secretion remain controversial. In hypothyroidism, impaired glucose tolerance is present (29). In rat studies, in pancreatic glucose sensitive beta cells impaired insulin response was determined. This condition was described as abnormalities in glucose transporter-2 (GLUT-2) protein levels and glucokinase activities that play a role in glucose transport in beta cells (29). However, in other rat studies, any impairment in insulin response was not determined (30). In our study, although there was not a significant difference between the patients with the diagnosis of hypothyroidism and the control group regarding insulin levels, there was a positive correlation between insulin and salusin levels in treated and untreated patient groups. This condition brings a question mark about the possible negative metabolic effects of salusin and necessitates large scaled studies among patients with metabolic syndrome to answer this question.

Abnormal lipid profile suggesting

hypercholesterolemia, especially increased triglyceride levels, increased total cholesterol/HDL ratios and increased LDL-Cholesterol/HDL-Cholesterol ratios are expected in patients with hypothyroidism (31). Decrease in LDL receptors in the liver causes a decrease in fractional clearance of LDL in hypothyroidism related to the decrease in CETP and hepatic lipase activities in the liver that are regulated by thyroid gland hormones. Increased insulin levels, hypertension and obesity among middle aged women also increase the risk. In this study, HDL-cholesterol levels were statistically significantly lower in hypothyroidism group compared with control cases while there was no statistically significant difference between the three groups regarding LDL-cholesterol and triglyceride levels that may be related to young age of participants, absence of smoking or whether on treatment or not.

Increase in hsCRP levels also increases cardiovascular disease risk. hsCRP is a stable marker of the inflammatory process with its half-life of about 19 hours (32). Half-life of hsCRP is longer than other cytokines and since its levels do not show a circadian variation, it protects its stability (33). It has been shown that in middle aged women, though in normal limits, mild elevations in hsCRP levels produce a pre- or pro-inflammatory condition associated with metabolic syndrome. In hypothyroidism, the presence of increased hsCRP levels with increased cardiovascular risk was also shown. In the same study, though hsCRP levels were increasing significantly in overt hypothyroidism, there was not any determined correlation between hsCRP and TSH levels (34). Another study showed increased CRP levels in patients with hypothyroidism compared to control group. Regression was also reported with L-thyroxin treatment (35). In our study, hsCRP levels were significantly higher in patients with hypothyroidism compared to control cases. However, there was not any significant positive effect of L-thyroxin treatment on sCRP levels. Salusin-alpha levels were not correlated with hsCRP levels but found to correlate positively with age, triglyceride and LDL cholesterol levels as expected in patients with hypothyroidism. Thus, it may be suggested that high hsCRP levels may play a role in the decrease in serum salusin-alpha secretion.

Another cytokine, TNF-alpha, is produced in macrophage, monocyte, epithelial cells and lymphocytes and it increases the production of interferon gamma (36). It is the major mediator molecule of apoptosis, inflammation and many autoimmune diseases (37). It also plays an important role in coronary artery disease

and plaque destabilization (38). In a study of August *et al.*, mRNA levels of TNF- α were determined to be higher in thyroid tissues of patients with autoimmune thyroiditis compared with control cases (39). Similarly in another study, in rats with experimental autoimmune thyroiditis, TNF- α levels with TNF antibodies were found in low levels in order to reverse the inflammation and to inhibit fibrosis (40). Hence, it may be told that TNF- α plays a role in fibrosis. In another study of Drugarin *et al.*, as well as other inflammatory markers, TNF- α levels were higher compared with control cases (41). In the study of Diez *et al.*, elevated TNF- α levels and TNF- α receptors were determined in patients with hypothyroidism compared with control cases (42). In our study, there was not a significant difference between treated or untreated patients and control cases regarding serum TNF- α levels and TNF- α levels and salusin levels were not found correlated. Even though TNF- α is an inflammatory marker playing a role in thyroid dysfunction, our lack of determining increased TNF- α levels may be associated with cross sectional design of our study, factors like age or gender of patients, period of disease, and laboratory changes due to activation-remission periods of hypothyroidism.

In conclusion, decreased levels of salusin alpha in hypothyroidism may be potentially associated with cardiovascular disease development and it could be considered as a marker in the progression of these diseases. Thus, in this study, decreased levels of salusin alpha in chronic diseases like hypothyroidism support its potential role in atherosclerosis development. The factors affecting salusin alpha secretion and its associations with other inflammatory markers would be clarified in future studies.

Conflict of interest

The authors declare that they have no conflict of interest concerning this article.

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