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Investigation of Serum NO, ADMA and Apelin Levels in Thyroid Dysfunction

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Abstract
Thyroid gland diseases are among the most common endocrine diseases and still continue to be an important health problem especially in developing countries. It was aimed to investigate serum NO, ADMA and Apelin levels in patients with thyroid dysfunction. This study was conducted with 150 thyroid patients and 50 healthy subjects. Study subjects were divided into three groups; control (n=50), hyperthyroid (n=75) and hypothyroid (n=75). Serum TSH, FT3, FT4 levels were measured by chemiluminescence method NO level were measured by spectrophotometric method, ADMA and apelin levels were measured by ELISA. Serum NO levels were higher in hypothyroid group than in hyperthyroid group, and the difference was statistically significant. Serum ADMA levels of the hyperthyroid group were significantly higher than the other two groups and the difference was statistically significant. The levels of serum apelin were statistically significantly higher in the hyperthyroid group than the other two groups. In patients with hyperthyroidism, ADMA and Apelin levels were higher, while NO level was lower. However, NO level was higher in patients with hypothyroidims than the other two groups. Apelin, which has been emphasized as a preventive and therapeutic agent particularly for the cardiovascular system, might have increased in hyperthyroid patients, regardless of NO, to protect cardiovascular system from possible adverse effects of ADMA.
Keywords: Asymmetric dimethylarginine, apelin, nitric oxide, thyroid dysfunction
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1. Introduction

Thyroid gland diseases are among the most common endocrine diseases and still continue to be an important health problem especially in developing countries (Arıkan et al.2007; Demers and Spercer 2003). Thyroid diseases may be classified as hyperthyroidism, hypothyroidism, subclinical hyperthyroidism and subclinical hypothyroidism, according to the level of thyroid hormones. Thyroid diseases may lead to endothelial dysfunction; however, the mechanism underlying the endothelial dysfunction in thyroid disease is not clear yet (Gürel et al. 2015; Obregon 2014). In addition, in case of hypothyroidism during which thyroid hormones are less secreted and in case of hyperthyroidism during which thyroid hormones are excessively secreted, the effects of these hormones on lipid metabolism and cholesterol levels continue to be the subject of various studies (Stephan et al. 2001; Ana et al. 2001).

In recent years, the thyroid dysfunction which is not sufficiently treated has been reported to be among the most common underlying causes of cardiovascular diseases. Besides, it has been stated that the timely identification of cardiac symptoms and effective treatment are required in patients with thyroid dysfunction (Gerdes et al. 2010; Galli et al. 2010; Biondi et al. 2010). It has been reported that the early diagnosis of the pathological findings of cardiovascular diseases in patients with thyroid dysfunction can make significant contributions in the prevention of the development, prognosis and treatment of endothelial dysfunction and heart failure (Siu et al. 2007; Bernadette 2012). The endothelial which is the main regulator of vascular homeostasis provides the balance between vasoconstriction and vasodilatation, smooth muscle cell proliferation and the inhibition and stimulation of migration, thrombogenesis, and fibrinolysis. However, endothelial dysfunction occurs if this balance is disrupted. The disruption of the endothelial function is the earliest symptom which is the sign of coronary artery disease (Kinlay et al.2001).

The nitric oxide (NO) which is the major vasodilator secreted from endothelium is synthesized from endogenic L-arginine by the nitric oxide synthase (NOS) enzyme system in many tissues in the body. NO is the regulatory molecule that functions both intracellularly and extracellularly (Albecht et al.2003). NO, one of the molecules playing the main role in the regulation of vascular tone, may be affected by these function changes in the cardiovascular system and play a role in the pathophysiology of these changes (Quesada et al. 2002). The activity of NOS enzyme, which is responsible for the synthesis of NO, is regulated by the asymmetric dimethylarginine (ADMA) molecule which is an endogenous inhibitor. The fact that ADMA is the major inhibitor

of NO biosynthesis has been demonstrated in many studies (Khazan et al. 2015; Chan et al.2002). It is considered that the ADMA increase that causes imbalances in the path of NO synthase has a significant role in atherogenesis by causing endothelial dysfunction (Böger 2003).

Apelin, the physiological effects of which are largely unknown, which is alleged to be associated with the cardiovascular system and NO, has been defined as a vasoactive peptide which is an endogenous ligand of the angiotensin-like receptor (APJ) (Ines et al. 2005; Szokodi et al. 2002). Some cardiovascular functions of Apelin/APJ system have been identified such as endothelium- related vasodilation, direct effect on the smooth muscle and vasoconstriction. In addition, apelin has been suggested to cause endothelium-related vasodilation by means of NO. Apelin has been reported to loosen the vascular and muscular structure by supporting the phosphorylation and activation of endothelial nitric oxide synthase (eNOS) that increases NO secretion (Tatemoto et al. 2001; Kleinz and Davenport 2005). It is not yet clear if there is a relationship between the blood levels of apelin and thyroid hormones, and if thyroid hormones have an effect on apelin levels, although both apelin and thyroid hormones play important roles in metabolism. From this point of view, it is worth investigating if apelin levels change in thyroid disorders (Gürel et al.2015).

The aim of our study is to provide an insight on the etiopathogenesis of the relationship between thyroid dysfunction and cardiovascular diseases which has not been fully clarified yet by investigating the serum NO, ADMA and apelin levels in thyroid dysfunction posing a significant risk for cardiovascular diseases.

2. Material and Methods

2.1. Study Group and Patient Selection

This prospective study was carried out on the patients with hyperthyroidism and hypothyroidism aged between 18 - 60 who had not received a treatment for the thyroid disease in the past three months and applied to internal diseases, surgery and goiter polyclinic in Rize Fındıklı Goitre Research Center and Artvin State Hospital between April-December 2012. Those who had used antihyperlipidemic drug in the past three months, those with impaired glucose tolerance and impaired fasting tolerance reflecting the known diabetes or insulin resistance, those who used drugs affecting the insulin and glucose metabolism (steroids, metformin, etc.) and those who used heart and blood pressure drugs were not included in the study.

In addition, those who were followed due to renal dysfunction and malignancy, those with known liver disease and those with immunological and chronic infectious diseases were also not included in the study. The people in the control group which was formed for the comparison were selected from healthy people who also applied to the same polyclinics for general control, did not have the apparent disease and did not use drugs regularly for any reason. A total of 200 people including 150 thyroid patients, 75 of them were diagnosed with hyperthyroidism (graves, subclinics hyperthyroidism, toxic nodular goiter) and 75 of them were diagnosed with hypothyroidism (Hashimato thyroid simple diffuse goiter, subclinical hypothyroidism) and 50 healthy individuals were included in the scope of the study. Individuals who were included in the study were divided into three groups as the control group (n=50), hyperthyroidism group (n=75) and hypothyroidism group (n=75). The study protocol was presented to "Atatürk University Faculty of Medicine, Non-Drug Clinical Research Ethics Committee", and approval was received with the protocol dated 27.04.2012 and numbered B.30.2.ATA./1. The "informed consent forms" were signed by all of the individuals who were included in the study by providing detailed information about the study. The study was planned and implemented in compliance with the Helsinki Declaration.

2.2. Blood sample collection and Demographic Data

In this prospective study, demographic data of patients such as age, gender and BMI were collected by face-to-face interview. In this prospective study, demographic data of the patients such as age, gender and BMI were collected by face-to-face interview. The data obtained were recorded in the study information form. Blood sample collection overnight fasting morning blood samples were obtained from each participants and collected from an antecubital vein. After centrifugation at 4000 x g for 10 minute, Thyroid-Stimulating hormone (TSH), free-triiodothyronine (FT3), free-tetraiodothyronine (FT4) and biochemistry parameters were studied. were studied in serum. For measurements of apelin, NO and ADMA, serum samples were stored at -80ºC until analysis.

2.3. Biochemical Analyses

Plasma TSH, FT3 and FT4 levels were determined by chemiluminescence using COBAS E-601 (Model E-Extensiyon, Serial No:0848-5, Japan) analyzer with Roche Diagnostik kits. Quantitative parameters of serum ADMA and apelin levels were measured by Enzyme Linked Immunosorbent Assay (ELISA) using (Bio-tek Power Wave –Xs Elısa, USA) ELISA test kits. The apelin results were stated as ng/ml. The ADMA results were stated as µmol/l. Serum NO level was measured by spectrofotometric method using of microplate reader with OxisResearch (Catalog No: 22110, Lot No:16527, INC) test kit. The NO results were stated as µM.

2.4. Statistical Analysis

Statistical analyses were performed using the SPSS 17.0. All variables were checked for normal distribution with Shapiro–Wilk test. One-way ANOVA with post-hoc Tukey test was applied for multiple comparison of normally distributed data. The values are presented as mean ± SD. Differences were considered significant at $p < 0.05$.

3. Result

There was a significant difference between the groups participating in the study in terms of average age. While the mean age of the control group was lowest, the average age of the hyperthyroid group was highest. These data are given in table 3.1.

The biochemical characteristics of the groups are summarized in Table 3.2. There were no significant difference in glucose, TG and fT3 levels between all the groups ($p>0.05$). Both TC and LDL-C levels were significantly higher in hyperthyroid and hypothyroid groups compared to control group ($p<0.01$), and also serum HDL-C concentrations were significantly lower than thyroid diseases groups ($p<0.05$). In hyperthyroid group, TSH levels were significantly lower compared to control ($p<0.05$) and hypothyroid groups ($p<0.01$). Patients with hypothyroid had higher TSH levels than to control and hyperthyroid groups ($p<0.05$). fT4 levels in subjects with hyperthyroid group were elevated compared to control and hypothyroid groups ($p<0.01$). There was no significant difference in serum fT4 levels between control and hypothyroid groups ($p>0.05$).

Serum NO levels were higher in hypothyroid group compared to control and hyperthyroid groups ($p<0.05$) (Fig 1). There was no significant difference in serum NO levels between control and hyperthyroid groups ($p>0.05$). Highest value of ADMA (Fig 2) and apelin (Fig 3) levels were observed in hyperthyroid group and statistically significant compared to control and hypothyroid groups ($p < 0.01$). There was no significant difference in serum apelin and ADMA levels between control and hypothyroid groups ($p>0.05$).

Table 3.1. Clinical and physical properties of control group, hyperthyroid group and hypothyroid group.

	Groups			
Values	Control (n=50)	Hyperthyroid (n=75)	Hypothyroid (n=75)	p
Age (Years)	34.76 ± 12.66 [b]	43.92 ± 9.06 [c]	39.48 ± 9.76[a]	p<0.05
Women (n),% Male (n),%	(n= 29), % 58 (n= 21), % 42	(n= 69), % 92 (n= 6), % 8	(n= 74), % 99 (n= 1), % 1	
BMİ (kg/m^2)	24.99 ± 4.67[b]	28.53 ± 4.95[a]	28.54 ± 5.67 [a]	p < 0.05

* Data are shown as mean ± mean standard deviation (OSS). Different superscripts in the same line show differences between groups ($p <0.05$).

Tablo 3.2. Biochemical characteristics of the groups.

	Groups			
Values	Control (n=50)	Hyperthyroid (n=75)	Hypothyroid (n=75	P
TC (mg/dl)	155.34 ± 55.77[a]	195.92 ± 55.25[c]	195.37 ± 49,37c	p<0.01
Glucose (mg/dl)	90.62 ± 45.13[a]	94.59 ± 20.27[a]	90.07 ± 16.66[a]	p>0.05
HDL-C (mg/dl)	35.50 ± 9.56[a]	43.01 ± 10.52[b]	42.61 ± 13.07[b]	p< 0.05
LDL-C (mg/dl)	85.38 ± 43.44[a]	122.50 ± 40.86[c]	118.37 ± 42.07[c]	p<0.01
TSH (µlU/ml)	1.72 ± 1.04[a]	1.15 ± 2.02[a]	2.71± 3.25[b]	p<0.05
FT3 (pg/ml)	2.99 ± 0.46[a]	2.99 ± 0.56[a]	2.85± 0.50[a]	p>0.05
FT4 (ng/dl)	1.27 ± 0.34[a]	3.68 ± 2.27[c]	1.20±0.28[a]	p<0.01

Abbreviations: HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; TC, total cholesterol; TG, triglyceride, TSH, Thyroid-Stimulating Hormone; FT3, free-triiodothyronine; FT4, free-tetraiodothyronine

*All values are mean±SD.a,b,c Means in the same row with different superscripts differ significantly ($p>0.05$, $p < 0.05$, $p<0,01$)

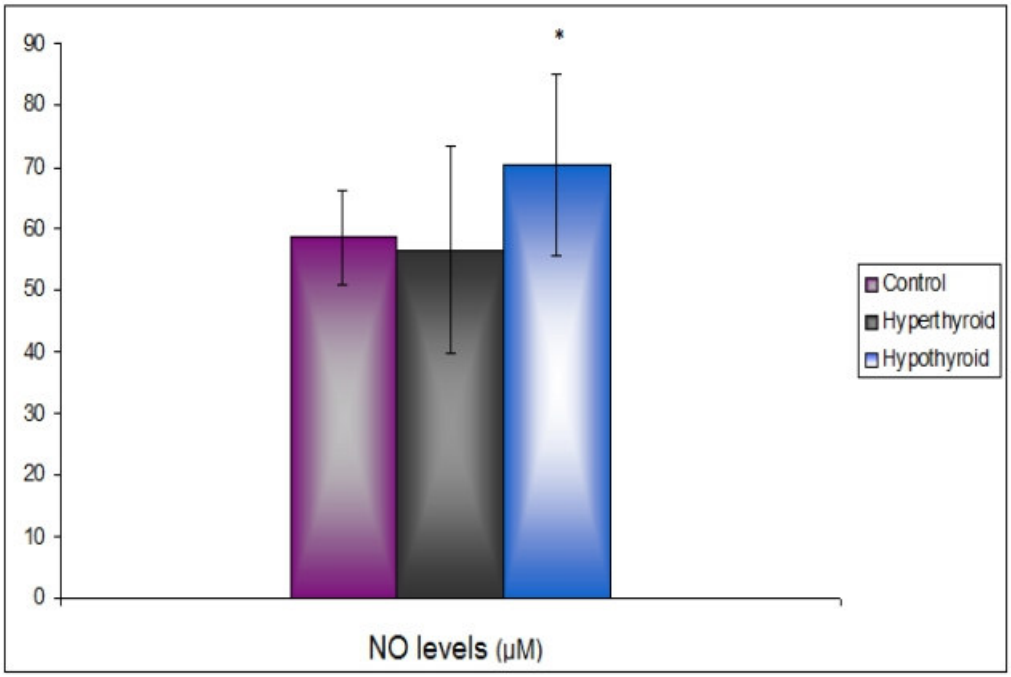

Figure 1. NO levels of the groups.
* Means in the same row with different superscripts differ significantly $p < 0.05$

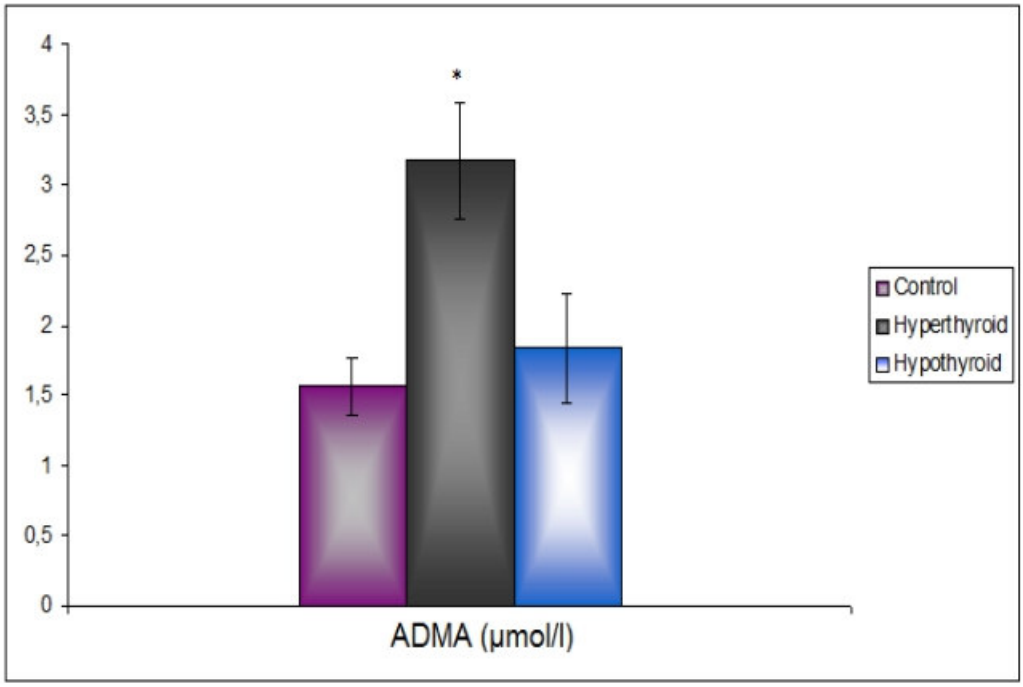

Figure 2. ADMA levels of the groups.
* Means in the same row with different superscripts differ significantly $p < 0.01$

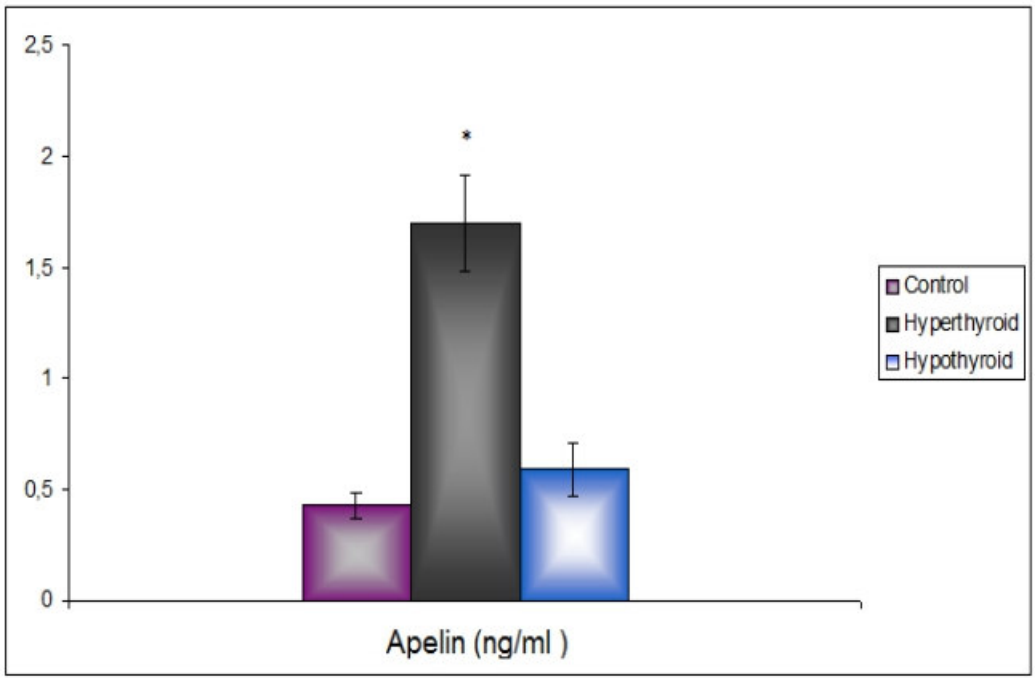

Figure 3. Apelin levels of the groups.
* Means in the same row with different superscripts differ significantly $p < 0.01$

Discussion

Thyroid dysfunction has been an important health problem for many years all over the world without decreasing its prevalence. Thyroid dysfunction is an important picture which has negative effects on the quality of life and life expectancy of individuals along with its all signs and symptoms and threatens many systems by progressing when its diagnosis and treatment are delayed. It has been determined by many studies that the cardiovascular system is one of the most important systems affected by thyroid dysfunction. The early diagnosis and treatment of cardiovascular diseases, one of the important symptoms and complications in thyroid dysfunction, are particularly important.

The changes in the thyroid hormone levels in both directions may affect metabolic, physiological and biochemical processes, and almost all systems of the body (Gürel et al.2015). Many studies have been carried out on the effects of thyroid dysfunction on the cardiovascular system. However, the mechanisms of the negative effects formed in thyroid dysfunction could not have been illuminated completely (Dillman 2010; Klein and Ojamaa 2001). Therefore, the amounts of many substances with the effects on the cardiovascular system in thyroid dysfunction have been discussed in some studies (Arikan et a. l 2007; Hemenegildo et al.2002). The regular and sufficient synthesis of NO, one of these substances, is important for many systems in the body. ADMA, which is the competitive inhibitor of NOS enzyme that allows the realization of NO synthesis, has an important place in the control of NO secretion. The increase in the amount of ADMA causes changes in the amount of NO. Especially in cases in which the amount of ADMA increases, some changes are observed in the cardiovascular system because NO level decreases (Tran et al. 2003; Böger and Ron 2005). Hermenegildo et al.(2002) investigated the plasma NO and ADMA levels in their study carried out on patients with thyroid dysfunction (Hemenegildo et al.2002). They reported that although NO levels were determined at a lower level in the hyperthyroidism group compared to the hypothyroidism group and the control group, ADMA level was found to be higher in the hyperthyroidism group compared to other two groups. In another study carried out by Arıkan et al. (2007) it was reported that NO level was at a significantly lower level in the hyperthyroidism group compared to the hypothyroidism and control groups, but ADMA level was at the higher level both in the hyperthyroidism and hypothyroidism groups compared to the control group (Arikan et a. l 2007). In our study, in line with the literature, serum NO level was higher in the hypothyroidism group compared to the serum NO level in the hyperthyroidism group, and the difference between them was statistically significant. In addition, serum ADMA levels in the hyperthyroidism group werefound to be statistically significantly higher than the ADMA levels of the other two groups.

In this study, decreased NO and free oxygen radicals that increased in the case of hyperthyroidism can be the most important reasons for the increase in ADMA level in patients with hyperthyroidism. Furthermore, the relationship of thyroid diseases with the protein metabolism may affect the synthesis of ADMA which is the derivative of a methylarginine. ADMA and NO are very effective on the cardiovascular system (Albecht et al.

2003; Herman and Moncada 2005). The reduction of the amount of NO which is an important vasodilator is described as one of the earliest symptoms indicating the endothelial dysfunction. One of the most important factors that affect the amount of NO is the decrease in the amount of eNOS which is involved in NO synthesis (Tousoulis et al. 2012; Kampoli et al. 2012; Miyazaki et al.1999). ADMA is the competitive endogenous inhibitor of eNOS. The increase in ADMA level decreases the amount of NO and causes vasoconstriction in the arteries (Böger and Ron 2005; Vallance and Leiper 2004).

Apelin is an important ring of this chain which is effective on the cardiovascular system. Serum apelin levels give important clues for the cardiovascular system. It has been reported by the studies carried out in recent years that apelin has significant effects on NO-dependent vasodilatation and on the cardiovascular system with some independent mechanisms (Kleinz and Davenport 2004; Mitra et al. 2006).

In their study carried out in 2015, Gürel et al. (2015) examined the serum apelin levels in patients with thyroid dysfunction and found serum apelin levels higher than the control group (Gürel et al.2015) . Apelin receptors have been detected in the thyroid gland. Therefore, changes in the thyroid hormones and TSH may affect the release of adipocytokines, so there is a possible relationship between thyroid status, thyroid dysfunction and adipocytokines (Gueorguiev et al. 2001).

5.Conclusions

As the result, in our study, serum apelin levels were also found to be higher both in the hypothyroid and hyperthyroidism group compared to the control group. The fact that the average serum NO level was low but the amount of apelin was high in the hyperthyroidism group can be an important finding that should be investigated. The effects of apelin on many systems are still the subject of research and study, and there are differences in the results obtained. We believe that more clinical and experimental scientific studies should be conducted on this subject.

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References

Albecht E, Stagemen C, Heeringa P, Henning R, Van Goor H. (2003) Protective role of endothelial nitric oxide synthase. *The Journal of Pathology,* 199, 8- 17.

Ana C, Phillipe B, Caroline V, Eric D, Frederique D, Michel B. (2001). Hepatic lipogenesis and cholesterol synthesis in hyperthyroid patients. *Journal of Endocrinology and Metabolism,* 86, 5353- 5357.

Arikan E, Karadag CH, Guldiken S. (2007). Asymmetric dimethylarginine levels in thyroid diseases. *Journal of Endocrinol Investigation,* 30 (3), 186- 191.

Bernadette B. (2012). Heart failure and thyroid dysfunction. *Europan Journal of Endocrinology,* 167, 609– 618.

Biondi B, Kahaly GJ. (2010). Cardiovascular involvement in patients with different causes of hyperthyroidism. *Nature Review Endocrinol,* 8, 431– 443.

Böger RH. (2003).The emerging role of ADMA as a novel cardiovascular risk factor. *Cardiovascular Research*, 59, 824-833.

Böger RH, Ron ES. (2005). L-Arginine improves vascular function by overcoming deleterious effects of ADMA, a novel cardiovascular risk factor. *Alternative Medicine Review,* 10, 14- 23.

Chan NN, Chan JC. (2002).Asymmetric dimethylarginine (ADMA): a potential link between endothelial dysfunction and cardiovascular diseases in insulin resistance syndrome? *Diabetologia*, 45, 1609- 1616.

Demers LM, Spencer CA. (2003). Laboratory Medicine Practice Guidelines: Laboratory Support for The Diagnosis and Monitoring, *Thyroid. Clin Endocrinol* (Oxf), 13, 57-67.

Dillmann W. (2010). Cardiac hypertrophy and thyroid hormone signaling. *Heart Failure Reviews,* 15, 125- 132.

Galli E, Pingitore A, Iervasi G. (2010). The role of thyroid hormone in the pathophysiology of heart failure: clinical evidence. *Heart Failuer Review.* 15, 155– 169.

Gerdes A.M & Iervasi G. (2010). Thyroid replacement therapy and heart failure. *Circulation,* 122, 385– 393.

Gueorguiev M, Goth ML, Korbonits M. (2001). Leptin and puberty: A review. *Pituitary,* 4, 79-86.

Gürel A, Doğantekin A, Özkan Y, Aydın S. (2015). Serum apelin levels in patients with thyroid dysfunction. *International Journal of Clinical Experimental Medicine,* 8(9),16394-16398.

Hemenegildo C, Medina P, Peiro M, Segarra G, Vıla JM, Ortega J and Lluch S. (2002). Plasma concentration of asymmetric dimethylarginine, an endogenous inhibitor of nitric oxide synthase, is elevated in hyperthyroid patients. *The Journal of Clinical Endocrinology &Metabolism,* 87, 5636- 5640.

Herman AG, Moncada S. (2005). Therapeutic potential of nitric oxide donors in the prevention and treatment of

atherosclerosis. *European Heart Journal*, 26, 1945- 1955.

Ines FP, Moreıra FL. (2005). Apelin: A novel neurohumoral modulator of the cardiovascular system. Pathophysiologic importance and potential use as a therapeutic target do aparelho cardiovascular. *Revista Portuguesa de Cardiologia,* 24, 1263- 1276.

Kampoli AM, Tousoulis D, Tentolouris C, Stefanadis C. (2012).Novel agents targeting nitric oxide. *Current Vascular Pharmacology,* 10, 61- 76.

Khazan M, Mehdi H. The Role of Nitric Oxide in Health and Diseases. (2015). *Scimetr,* 3(1), 20987.

Kinlay S, Libby P, Ganz P. (2001). Endothelial function and coronary artery disease. *Current Opinion in Lipidology*, 12, 383- 389.

Kleinz M, Davenport AP. (2005). Emerging roles of Apelin in biology and medicine. *Pharmacology & Therapeutics,* 107, 198- 211.

Klein I, Ojamaa K. (2001). Thyroid hormone and cardiovascular system. *The New England Journal of Medicine,* 344, 501- 509.

Kleinz MJ, Davenport AP. (2004). Immunocytochemical localization of the endogenous vasoactive peptide Apelin to human vascular and endocardial endothelial cells. *Regulatory Peptides*, 118, 119- 125.

Mitra A, Katovich MJ, Mecca A, Rowland NE. (2006). Effectsof central and peripheral injections of Apelin on fluid intake and cardiovascular parameters in rats. *Physiology &Behavior*, 89, 221- 225.

Miyazaki H, Matsuoka, Cooke JP. (1999). Endogenous nitric oxide synthase inhibitor: a novel marker of atherosclerosis. *Circulation,* 99, 1141- 1146.

Obregon MJ. (2014). Adipose tissues and thyroid hormones. *Front Physiol,* 5, 479-480.

Quesada A, Sainz J, Wangensteen R, Rodriguez- Gomez I, Vargas F, Osuna A. (2002). Nitric oxide synthase activity in hyperthyroid and hypothyroid rats. *Europan Journal Endocrinology,* 147(1), 117-22.

Siu CW, Yeung CY, Lau CP, Kung AW, Tse HF. Incidence, clinical characteristics and outcome of congestive heart failure as the initial presentation in patients with primary hyperthyroidism. (2007). *Heart,* 93, 483– 487.

Stephan J, Bakker L, Ter Maaten j, Corrie P.S. Joris PJ, Slates R, Rijk OB. (2001), The relationship between thyrotropin and low density lipoprotein cholesterol is modified by insulin sensitivity in healthy euthyroid. *The Journal of Endocrinology and Metabolism,* 86, 1206- 1211.

Szokodi I, Tavi P, Foldes G, Voutilainen-Myllyla S, Ilves M, Tokola H, Pikkarainen S, Piuhola J, Rysa J, Toth M, Ruskoaho H. (2002). Apelin, the novel endogenous ligand of the orphan receptor APJ, regulates cardiac contractility. *Circulation Research,* 91, 434- 440.

Tatemoto K, Takayama K, Zou MX, Kumaki I, Zhang W, Kumano K, Fujimiya M. (2001). The novel peptide Apelin lowers blood pressure via a nitric oxide-dependent mechanism. *Regulatory Peptides,* 99, 87- 92.

Tousoulis D, Kampoli AM, Tentolouris C, Papageorgiou N, Stefanadis C. (2012). The role of nitric oxide on endothelial function. *Current Vascular Pharmacology,* 10, 4- 18.

Tran CT, Leiper JM, Vallance P. (2003). The DDAH/ADMA/NOS pathway. *Atheroscler Suppl*, 4, 33- 40.

Vallance P, Leiper J. (2004). Cardiovascular biology of asymmetric dimetylarginine: dimethylarginine dimethylaminohydrolase pathway. *Arteriosclerosis, Thrombosis and Vascular Biology,* 24, 1023- 1030.