ARAŞTIRMA MAKALESİ

COMPARISON OF SCD40L LEVELS IN PATIENTS WITH SUBCLINICAL HYPOTHYROIDISM BEFORE AND AFTER LEVOTHYROXINE TREATMENT

SUBKLİNİK HİPOTROİDİLİ HASTALARDA LEVOTİROKSİN TEDAVİSİ ÖNCESİ VE SONRASI SCD40L DÜZEYLERİNİN KARŞILAŞTIRILMASI

Ferda Bilgir[1], Oktay Bilgir[2], Mehmet Calan[3], Ozlem Gursoy Calan[4], Arif Yuksel[2]

[1]Katip Celebi University Medical School, Ataturk Training and Research Hospital Department of Allergy and Immunology, Izmir

[2]Izmir Bozyaka Training and Research Hospital, Department of Internal Medicine, Izmir

[3]Dokuz Eylul University Medical School, Department of Endocrinology and Metabolism, İzmir
[4]Dokuz Eylul University Medical School, Department of Biochemistry, Bozyaka, Izmir

ABSTRACT
The aim of the study is to determine sCD40L levels which is the sign for inflammation, thrombosis, atherosclerosis in common subclinical hypothyroidism (SHO) and if there is change in sCD40L levels after levothyroxine treatment.
Thirty-two patients diagnosed with subclinical hypothyroidism and 30 healthy control group is enrolled in the study. In patients with SHO, blood samples were re-collected after a 3 months treatment of levothyroxine. TSH, FT3, FT4 and sCD40L levels were determined and levels were compared between groups.
sCD40L levels were found significantly high in SHO patient group than control group (p=0.0001). Increase in sCD40L levels are observed after 3 months treatment of 50 µg/day levothyroxine but this result was not statistically significant (p=0.587). The relation between sCD40L levels and platelet count were analysed with Pearson Correlation analysis but no significant correlation could be determined. (t= -0,07, p=0,955). As sCD40L levels are found to be high in SHO patients compared with healthy control group, and continuation of being high after levothyroxine treatment for 3 months makes us think that, the hormone replacemant therapy does not reduce the risk of disease and new treatment strategies should be improved.
Keywords: Subclinical hypothyroidism, sCD40L, levothyroxine

Corresponding author:
Assoc.Prof.Dr.Oktay Bilgir, MD
E-mail:oktaybilgir@gmail.com
Tel.:+902322505050/5022;
fax: +902322614444

ÖZET
Çalışmanın amacı Subklinik Hipotroidide (SHO) inflamasyon, tromboz ve ateroskleroz işareti olan sCD40L düzeyinin belirlenmesi ve levotiroksin tedavisi ile değişip değişmediğini göstermektir.
Subklinik Hipotroidi tanısı alan 32 hasta ile 30 sağlıklı kontrol grubu çalışmaya alındı. SHO'lu hastaların 3 ay levotiroksin tedavisi aldıktan sonra kan örnekleri tekrar alındı. TSH,FT3,FT4 ve sCD40L düzeyleri belirlenerek gruplar arası karşılaştırıldı.
sCD40L düzeyleri SHO hasta grubunda kontrol grubuna göre anlamlı ölçüde yüksek bulundu (p=0.0001) . sCD40Ldüzeyindeki artış 3 aylık 50µg/gün levotiroksin tedavisi verildikten sonrasında da gözlendi ancak bu sonuç istatistiksel olarak anlamlı değildi (p=0.587). sCD40Lile trombosit düzeyleri arasındaki ilgi Pearson Correlation ile analizi yapıldı ve anlamlı korelasyon yoktu (t= -0,07, p=0,955).
Sağlıklı kontrol grubu ile karşılaştırıldığında SHO'lu grupta sCD40L düzeyi yüksek bulundu ve 3 aylık levotiroksin tedavisi sonrasında da devam ediyordu, bu bize hormon replasman tedavisinin hastalık riskini azaltmadığını, yeni tedavi stratejileri geliştirilmesi gerektirdiğini düşündürmektedir.

INTRODUCTION
Subclinical hypothyroidism (SHO), characterized by high serum troid stimulant hormone level (TSH) and normal serum free tyroxin level, is a common disease in population. Prevalance in adults is about 1-10 % and 7-16 % in elderly population (1,2).
In recent years, it has been indicated that, interaction of CD40 and it's immunmodulating ligands (CD40L) causes immune, activation of vascular cells and platelets cause inflammation, atherosclerosis and thrombosis (3).
CD40 is a member of tumour necrosis factor superfamily and type 1 transmebmrane receptor protein (1). Basically, although B cells express CD40, other immune cells, epithelial cells, fibroblasts and platelets, endothelial cells and smooth muscle cells also express CD40 (4). While CD40 molecules appear on the surface of these cells, CD40L receptor is expressed at the same time. CD40 molecule is expressed by proinflammatory stimulants like interleukin-1,3,4 with interferon- γ and tumour necrosis factor α. CD40 expression is regulated by transcriptional factors like nuclear factor - kappa β and transcription kinases (5). Studies performed with endothelial cell culture shows that sCD40L is antiapoptotic, proliferative and proangiogenetic effective (6).
We planned this study to analyse sCD40L level and the potential effects of 3 months levothyroxine treatment on this level in commonly observed subclinical hypotiroidism cases.

MATERIAL AND METHODS
In this study, 32 patients were enrolled between 2009 and 2011 in İzmir Bozyaka Training and Research Hospital Internal Medicine Outpatient Clinic with TSH levels higher than 4.25 mIU/mL, and normal FT_3, FT_4 levels and diagnosed with SHO. Patients with any kind of treatment, thyroid surgery or radioactive iode treatment, history of any chronic disease were defined as the exclusion criteria of the study and the patients enrolled to the study accordingly. Also, 30 healty volunteers were included to the study for the control group. SHO patients received 50 µg/day levothyroxine depending on the appropriate indication for 3 months. This study is initiated after the approval of İzmir Bozyaka Training and Reseach Hospital Ethics Committee.
10ml of venous blood samples were collected to non-anticoagulant tubes both from SHO patients and control group to determine the TSH, FT_3, FT_4 and sCD40L levels before and after treatment. The blood samples were stored at room temperatre for about 30 minutes until they coagulate. Then, they were centrifuged for 5 minutes in 4000 rpm. Serum TSH, FT_3, FT_4 levels were analyzed by 'Immulite 2000' device with chemiluminencence immunoassay method. Complete blood count (CBC) was determined by using A Cell-Dyn 3500 (Abbott).

Serum samples were frozen at - 80 °C in eppendorf tubes to determine the sCD40L levels. Serum sCD40L (Bender Med Systems Inc, Vienna, Austria), levels were determined by ELISA method according to manufacturer's instructions. The sensitivity of the assay was determined to be 0.06 ng/mL. The intra-assay coefficients of variation (CV) of sCD40L was 4% and inter-assay coefficients of variation was 6.8%.

Statistical analyses:

All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS, version 11.0 for Windows, Chicago, Ill, USA). Data were expressed as mean±standard deviation (SD). A "p" value < 0.05 was accepted as significant.

A nonparametric Mann-Whitney U test was used to compare the variables between patients and controls and Wilcoxon Signed Ranks Test was used to compare the variables between SHO patients who treated after and before. Pearson correlation analysis was applied and Pearson coefficient of correlation (r) was used to show the relationship between platelet count and sCD40L levels.

RESULTS

Mean sCD40L, platelet count, FT3, FT4 and TSH levels of SHO group, SHO patients after treatment and healthy controls are presented in table 1. There was a significant difference in sCD40L, FT3 and TSH levels between SHO patients and control group (p values are 0.000, 0.008, 0.000 respectively) (Figure 1 and 2).

More increase in sCD40L level was detected after 3 months treatment with 50 µg/day levothyroxine, whereas sCD40L values before levothyroxine treatment of patients with SHO are statistically significantly high, but this increase did not achieve statistically significant levels (p=0.587) (Figure 1).

To determine whether there is a correlation between levels of sCD40L and platelet counts, the results of correlation analysis and regression analysis are evaluated. Pearson correlation analysis revealed that platelet count was not correlated with sCD40L level (r =-0.07, p=0.955) (Figure 3).

There was a significant increase in sCD40L level between SHO and control groups (* p=0.000). But, no statistically significant difference was obtained between SHO group and patients after treatment (** p=0.587).

There was a significant difference in FT3 and TSH levels between SHO patients and control group (p values are 0.008, 0.0001 respectively). There was not a statistically significant difference bet ween FT3 levels before treatment and FT3 levels after treatment (p=0.116).

	SHO (before treatment)	SHO (after treatment)	Control group
sCD40L (ng/mL)	2.09±1.69	2.47±2.24	1.12±1.48
Platelet count (10^9/L)	277.31±124.68	252.95±82.73	228.43±54.24
FT3 (pg/mL)	3.02±0.43	2.84±0.45	2.69±0.38
FT4 (ng/dL)	0.82±0.13	0.83±0.17	0.81±0.11
TSH (mIU/mL)	6.57±2.53	4.64±2.99	2.11±1.26

Table1. Clinical parameters for SHO patients before and after treatment and healthy control group (mean±SD)

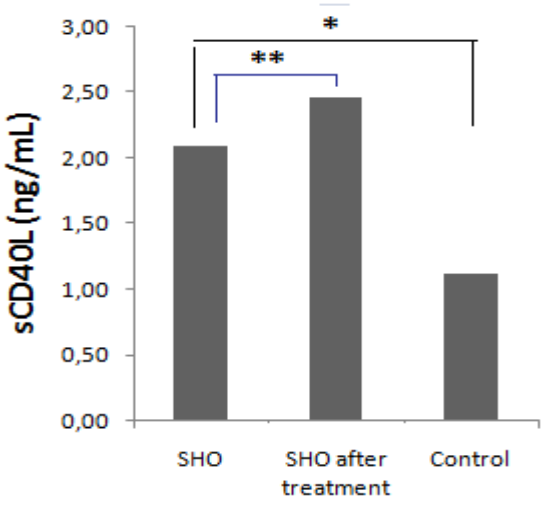

Figure 1. Comparison of SHO patients before and after treatment and TSH, FT3 and FT4 values of the healthy control group.

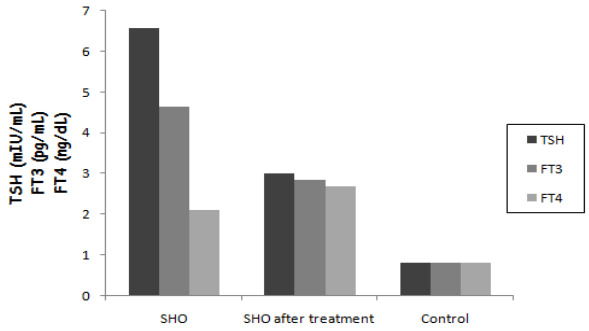

Figure 2. Comparison of values of TSH, FT3 and FT4 of patients with SHO patients before treatment, SHO patients after treatment and healthy control group.

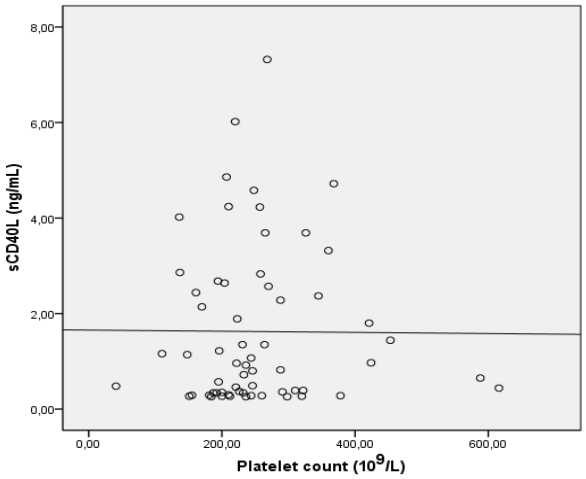

Figure 3. Correlation between platelet count and sCD40L level.

TSH levels were reduced as statistically significant after treatment were administered for the patients with SHO (p=0.000), but TSH levels reduced after treatment were still significantly higher in comparison with control group (p=0.001).

There was no significant correlation between platelet count and serum sCD40L level (r= -0.07, p=0.955).

DISCUSSION

Subclinical hypothyroidism is a common disease affecting 3 million people in England and 15 million people in USA. The importance of this disease is especially because of the existance of the data causing aterogenic lipid profile and endothel dysfunction. In addition, association of subclinical hypothyroidism with cardiovascular disease or the increase in mortality it caused has been reported at the least in several studies. The fact that this common disease causes serious consequences, increases the importance of the studies that were and will be conducted.

Molecule CD40 was identified on B cells for the first time and it was expressed in all development stages of B Cells and defined as an active B lymphocytes activation molecule functionally. (7,8,9). Therefore, it is thought that it plays an important role for potentiation of B Cells. CD40 molecule bonding to target cells creates costimulatuar alert which is necessary for B Cell proliferation, immunoglobulin class swiching, antibody production, prevention of germinal center B-cell apoptosis, sensitivity to maturation and formation of longlife term memory cells. (10,11,12,13).

It has been thought that CD40 remark cascade has role at soma auto immune diseases. It has been claimed that the antibody generated aganist CD154, which is ligand of CD40, provides improvement for diseases by blocking both primary and secondary immune response at expremental strong humoral component of lupus nephritis,myasthenia gravis and Graves disease. (14,15,16).

After the data obtained from experimental studies, CD40/CD40L interactions in acute croner syndromes were investigated in clinical trials. When compared with healthy controls in patients with acute myocardial infarction or unstable angina pectoris, it was determined that sCD40L level was significantly high in cirrulatory (18,19).

It has been thought for recent studies that in vascular wall CD40 has an impressive role on T lymphocytes with dendric cells interaction (20). There are evidences for activated dendric cells, which has role in formation of atherosclerotic plaques in carotis with coroner arteries, affected maturation by CD40L and maturate dendric cells also organized characterictic T lymphocyte infiltration in atheromatous plague. (21). Also, it leads atherogenesis by providing secretion of adipokines from adipocytes (22).

It has been shown that it has activated platelets by binding to sCD40L glycoprotein IIb / IIIa receptor and the sCD40L secretion is also decreased when secreted from active platelets when glycoprotein IIb/IIa receptor antagonist is used in vitro (17).

However it was shown in a study that CD40's overexpression induced inhibition of nitric oxide and platalet activation (23). It had been claimed that in another study of Subclinical hypothyroidism, low-grade inflammation occured and it also caused endothelial dysfunction and COX-2 mediated degradation use of nitric oxide due to increased oxidatative stress (24). These results are thought to be a risk for atherosclerosis and ischemic heart disease. We also believe that one of the most important occuring reasons as well as inflammation and atherosclerosis present in subclinical hypothyroidism is sCD40L which is increased with this disease.

In a prospective study in subclinical hypothyroidism, published previously, it was claimed that levothyroxine treatment reduced the cardiovascular risk and improved endothelial function (25). On the other hand, in a different study, it was considered that the use of levothyroxine could perform inhibition for innate immunity by reducing inflammatory cells, plaque cytokines and oxidative stress (26).

In our study, presence of higher sCD40L levels after levothyroxine treatment contrasts with studies performed previously. The role of CD40 molecule in inflammation, thrombosis and atherogenesis in patients with subclinical hypothyroidism is a known fact. Although presence of higher sCD40L level before treatment is not a statistically significant difference in comparison improvement of TSH levels after treatment, due to increasing rising of sCD40L level, we think that levothyroxine treatment should be reviewed in patients with subclinical hypothyroidism and treatment options for CD40 is to be considered.

REFERENCES

1) Tunbridge WMG, Evered DC, Hall R, et al. The spectrum of thyroid disease in a community: the Whickham survey. Clin Endocrinol 1977 ;7: 481–93.

2) Rosenthal MJ, Hunt WC, Garry PJ, et al. Thyroid failure in the elderly: microsomal antibodies discriminant for therapy. JAMA 1987;258:209–13.

3) Anand SX, Viles-Gonzalez JF, Badimon JJ, Cavusoglu E, Marmur JD. Membrane-associated CD40L and sCD40L in atherothrombotic disease. Thromb Haemost 2003;90:377– 84.

4) Schonbeck U, Libby P. The CD40/CD154 receptor/ligand dyad. Cell Mol LifeSci 2001; 58:4–43

5)Chen Y, Chen J, Xiong Y, et al. Internalization of CD40 regulates its signal transduction in vascular endothelial cells. Biochem Biophys Res Commun 2006;345:106 –17.

6)Deregibus MC, Buttiglieri S, Russo S, Bussolati B, Camussi G. CD40-dependent activation of phosphatidylinositol 3-kinase/Akt pathway mediates endothelial cell survival and in vitro angiogenesis. *J Biol Chem*.2003;278:18008 –14.

7) Paulie S, Rosen A, Ehlin-Henriksson B, Braesch-Andersen S, Jakobson E, Koho H, Perlmann P. The human B lymphocyte and carcinoma antigen, CDw40, is a phosphoprotein involved in growth signal transduction. J. Immunol 1989;142:590–5.

8) Ledbetter JA, Shu G, Gallagher M, Clark EA. Augmentation of normal and malignant B cell proliferation by monoclonal antibody to the B cell-specific antigen BP50 (CDW40). J. Immunol 1987;138:788–94.

9) Banchereau J, Bazan F, Blanchard D, Briere F, Galizzi JP, van Kooten C, Liu YJ, Rousset F, Saeland S. The CD40 antigen and its ligand. Annu. Rev. Immunol 1994;12:881–922.

10) Lane P, Traunecker A, Hubele S, Inui S, Lanzavecchia A, Gray D. Activated human T cells Express a ligand for the human B cell-associated antigen CD40 which participates in T cell-dependent activation of B lymphocytes. Eur. J. Immunol 1992;22:2573–8.

11) Jabara HH, Fu SM, Geha RS, Vercelli D. CD40 and IgE: synergism between anti-CD40 monoclonal antibody and interleukin 4 in the induction of IgE synthesis by highly purified human B cells. J. Exp.Med 1990;172:1861–4.

12) Callard RE, Smith SH, Herbert J, Morgan G, Padayachee M, Lederman S, Chess L, Kroczek RA, Fanslow WC, Armitage RJ. CD40 ligand (CD40L) expression and B cell function in agammaglobulinemia with normal or elevated levels of IgM (HIM). Comparison of X-linked, autosomal recessive, and non-X-linked forms of the disease, and obligate carriers. J. Immunol 1994;153:3295–306.

13) Liu YJ, Joshua DE, Williams GT, Smith CA, Gordon J, MacLennan IC. Mechanism of antigen-driven selection in germinal centres. Nature 1989;342:929–31.

14) Mohan C, Shi Y, Laman JD, Datta SK. Interaction between CD40 and its ligand gp39 in the development of murine lupus nephritis. J. Immunol 1995;154:1470–80.

15) Im SH, Barchan D, Maiti PK, Fuchs S, Souroujon MC. Blockade of CD40 ligand suppresses chronic experimental myasthenia gravis by down-regulation of Th1 differentiation and up-regulation of CTLA-4. J. Immunol 2001;166:6893–8.

16) Chen CR, Aliesky HA, Guo J, Rapoport B, McLachlan SM. Blockade of costimulation between T cells and antigen-presenting cells: an approach to suppress murine Graves' disease induced using thyrotropin receptor-expressing adenovirus. Thyroid 2006;16:427–34.

17) Nannizzi-Alaimo L, Alves VL, Phillips DR. Inhibitory effects of glycoprotein IIb/IIIa antagonists and aspirin on the release of soluble CD40 ligand during platelet stimulation. Circulation 2003;107:1123–8.

18) Antoniades C, Tousoulis D, Vasiliadou C, Stefanadi E, Marinou K, Stefanadis C. Genetic polymorphisms of platelet glycoprotein Ia and the risk for premature myocardial infarction: effects on the release of sCD40L during the acute phase of premature myocardial infarction. J Am Coll Cardiol 2006;47:1959–66.

19) Tousoulis D, Antoniades C, Nikolopoulou A, et al. Interaction between cytokines and sCD40L in patients with stable and unstable coronary syndromes. Eur J Clin Invest 2007;37:623– 8.

20) Pryshchep O, Ma-Krupa W, Younge BR, Goronzy JJ, Weyand CM. Vessel-specific toll-like receptor profiles in human medium and large arteries. Circulation 2008;118:1276–84.

21) Erbel C, Sato K, Meyer FB, et al. Functional profile of activated dendritic cells in unstable atherosclerotic plaque. Basic Res Cardiol 2007;102:123–32.

22) Poggi M, Jager J, Paulmyer-Lacroix O, et al. The inflammatory receptor CD40 is expressed on human adipocytes: contribution to crosstalk between lymphocytes and adipocytes. Diabetologia 2009;52:1152–63.

23) Schafer A, Wiesmann F, Neubauer S, Eigenthaler M, Bauersachs J, Channon KM. Rapid regulation of platelet activation in vivo by nitric oxide. Circulation 2004;109:1819 –22.

24) Taddei S, Caraccio N, Airdis A, Dardano A, Versari D, Ghiadoni L, Ferrannini E, Salvetti A, Monzani F. Low-Grade Systemi Inflammation Causes Endothelial Dysfunction in Patients with Hashimoto's Thyroiditis. J Clin Endocrinol Metab 2006; 91:5076-82.

25) Razvi S, Ingoe L, Keeka G, Oates C, McMillan C, Weaver JU. The beneficial effect of L-thyroxine on cardiovascular risk factors, endothelial function, and quality of life in subclinical hypothyroidism: randomized, crossover trial. J Clin Endocrinol Metab 2007;92:1715–23.

26)Marfella R, Ferrariccio F, Rizzo MR, Portoghese M, Barbieri M, Basilio C, Nersita R et al. Innate Immune Activity in Plaque of Patients with Untreated and L-Thyroxine-Treated Subclinical Hypothyroidism.J Clin Endocrinol Metab 2011;96:1015-20.

Yazının alınma tarihi:18.08. 2014
Kabül tarihi:28.09. 2014
Online basım:01.10 2014