

Serum IL-1 β , IL-6, IL-10 and TNF- α Levels in Thyroidectomized Rats ^[1]

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Abstract

This study was conducted to determine the serum levels of interleukin (IL)-1 β , IL-6, IL-10 and tumor necrosis factor alpha (TNF- α) in thyroidectomized rats. Hypothyroidism was performed by surgical thyroidectomy in rats. Four weeks after from surgical procedure, selected cytokines levels were evaluated. Hypothyroidism was confirmed by elevated thyroid stimulating hormone (TSH) and decreased free tri-iodothyronine (fT3) levels (P<0.05) in thyroidectomized group. Serum IL-1 β , IL-6, IL-10 levels have shown slight increase, whereas only TNF- α level (P<0.05) were significant statistically in thyroidectomized rats compared with control group. In conclusion, the obtained data suggest that elevated levels of cytokines could be as a consequence of thyroidectomy operation.

Keywords: Cytokine, Immune function, Rodent, Thyroid alteration, Trauma

Tiroidektomize Ratlarda Serum IL-1 β , IL-6, IL-10 ve TNF- α Seviyeleri

Özet

Bu çalışmada, tiroidektomize ratların serum interlökin (IL)-1 β , IL-6, IL-10 and TNF- α düzeylerinin belirlenmesi amaçlanmıştır. Hipotiroidizm cerrahi yolla tiroidektomi yapılarak gerçekleştirildi. Cerrahi işlemden sonraki 4. haftada, seçili sitokinlerin seviyeleri ölçüldü. Hipotiroidizm, tiroidektomize grubun tiroid uyarıcı hormon (TSH) düzeyindeki artış (P<0.05) ve serbest tri-iyodotironin (fT3) düzeyindeki azalış (P<0.05) ile teyit edildi. Tiroidektomize ratların serum IL-1 β , IL-6, IL-10 seviyelerinin kontrol grubuna kıyasla hafif düzeyde yükseldiği ancak, sadece TNF- α düzeyindeki artışın istatistikî olarak anlamlı (P<0.05) olduğu görüldü. Sonuç olarak elde edilen veriler, sitokin seviyelerindeki yükselmenin, tiroidektomi operasyonunun bir sonucu olabileceğini düşündürmektedir.

Anahtar sözcükler: İmmun fonksiyon, Rodent, Sitokin, Tiroid bozulması, Travma

INTRODUCTION

Cytokines are a heterogeneous group of polypeptides which have multifunctional act as modulating, triggering and regulating of inflammatory and immune responses. Most cytokines have autocrine and paracrine effects owing to multiple cellular sources. Interleukins are described as any of various compounds of low molecular weight (~17 to 60 kd) these are produced by lymphocytes, macrophages and monocytes and they act as regulate of the humoral and innate immune functions and inflammation cascades ^[1].

The firstly discovered interleukin was IL-1 which has extensive family consisted by 11 members. The major members of IL-1 family are IL-1 α and IL-1 β , these two proteins are binding to the same receptor complexes namely as

IL-1 type I receptor (IL-1RI) and IL-1RII due to exert similar biological effects act as proinflammatory cytokine which has potentiating immune and inflammatory responses Multifunctional, pleiotropic cytokine IL-6 is regulated of immune and acute-phase responses, hematopoiesis and inflammation which is produced by innate immune cells (T helper 2; Th2), monocytes and macrophages, endothelial cells, fibroblasts, that promotes T-cell proliferation, B-cell differentiation and survival triggering by IL-1 and tumor necrosis factor alpha (TNF- α). When firstly described in late of the 80's an anti-inflammatory cytokine IL-10 was known as cytokine synthesis inhibiting factor. Whilst the major source of this cytokine is macrophages, Th2 cells, monocytes and keratinocytes may also produce. Another pro-inflammatory cytokine tumor necrosis factor alpha (TNF- α) is in a relationship with the physiopathologies



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of cancer, neurological, cardiovascular, autoimmune and metabolic disorders through activation of nuclear factor kappa B (NF- κ B) pathway [2,3].

Thyroid hormones are affected nearly all metabolic processes by using different pathways. Hypothyroidism have described as absence or lacking of thyroid hormones which could cause of abnormalities on metabolic and immunological functions. Cytokines play pivotal role in autoimmune thyroid disorders namely as Graves' disease or Hashimoto's thyroiditis [4]. Various models have been widely used as congenital hypothyroid animals due to thyroid gland dysgenesis or thyroid dyshormonogenesis, thyroid hormone receptor (TR) gene mutated animals, and thyroid hormone transport or metabolism modified animals for enhancing knowledge and clarify the thyroid hormone action [5]. In order to realize adult-onset hypothyroidism in rodents, thiourea based selenium analogue antithyroid agents have been used in such as propylthiouracil (PTU) or methimazole (MMI). Nevertheless, previously studies revealed that PTU and MMI have immunomodulatory effects [6].

Therefore, hypothyroidism was induced by thyroidectomy operation due to withdrawn adverse effects of antithyroid drugs on immune system function and it was aimed to determine the serum levels of IL-1, -6, -10 and TNF- α in adult-onset hypothyroidism.

MATERIAL and METHODS

Animals

Ten male Wistar rats (12 weeks age) obtained from Selcuk University Experimental Medicine Research and Application Center. Rats were kept in a room at a constant temperature 22 \pm 2°C with 50% relative humidity, 12 h light/dark cycle period and housed in polycarbonate cages with fed by standart rat chow and tap water *ad libitum*. All experimental procedure was approved by Selcuk University Experimental Medicine Research and Application Center Local Ethics Committee (Approval number: 2014/16).

Experimental Protocol

Rats were randomly divided into two groups as control (n=5) and thyroidectomized (Tx, n=5). Hypothyroidism was generated by surgical thyroidectomy in rats with anesthetized by xylazine HCl (10 mg/kg/BW) and ketamine HCl (50 mg/kg/BW). Briefly, using a stereomicroscope (Olympus Co.,Tokyo, Japan) for better observation, the stenothyroid muscle was cut and the trachea was exposed. The parathyroid gland was found, dissected from the thyroid gland, and implanted into the surrounding neck muscle. The thyroid gland was completely excised. After surgery, carprofen (Rimadyl® Pfizer, 5 mg/kg) was injected over 3 days to alleviate pain. The control group received the pre- and post-surgery treatment. Four weeks after

thyroidectomy operation blood samples were taken by cardiac puncture under deeply anesthetized with ketamine HCl and xylazine HCl collected into non-coagulant tubes and centrifuged (3.000 x g for 5 min) after that collected sera was stored -80°C until analysis.

Assessment of Thyroid Hormones and Cytokines

Serum concentrations of thyroid stimulation hormone (TSH) and free tri-iodothyronine (fT3) were determined by autoanalyser (ADVIA Centaur XP Immunoassay System, Siemens, USA) and selected cytokines IL-1 β (Cat. No. BMS630), IL-6 (Cat. No. BMS625) and IL-10 (Cat. No. BMS629) and TNF- α (Cat. No. BMS622) were determined by ELISA (Bio-tek Instruments, Inc.) using sandwich enzyme-linked immunosorbent method according to manufacturer's (Ebioscience) instructions.

Statistical Analysis

Statistical analysis was performed with the SPSS 19.0 package program for Windows (SPSS, Inc., Chicago, IL, USA). Data are expressed as mean \pm standard error of the mean (SEM) Student's t-test was used for determination among the groups. P<0.05 was considered for statistically significant.

RESULTS

As shown as *Table 1*, hypothyroidism was confirmed by elevated TSH and decreased fT3 levels (P<0.05) in thyroidectomized group.

According to obtained data IL-1 β , IL-6 and IL-10 levels (*Table 2*) were slightly increased but these were not statistically significance except TNF- α level in thyroidectomized animals compared with control group (P<0.05).

Table 1. Tiroid hormon düzeyleri (mean \pm SEM, n=5)		
Table 1. Thyroid hormone levels (mean \pm SEM, n=5)		
Group	TSH μ U/mL	fT3 ng/dL
Control	1.73 \pm 0.29 b	3.36 \pm 0.27 a
Tx	19.54 \pm 1.61 a	2.03 \pm 0.32 b

a,b Different letters in the same column refers the differences between the groups (P<0.05)

DISCUSSION

Hypothyroidism could cause of reduction in serum pro-inflammatory cytokines IL-6 and TNF- α levels in mice [7], rats [8], which were induced by PTU, MMI and thyroidectomy. Also, Kızıltunc et al.[9] reported that IL-6 and TNF- α serum levels were decreased in humans who suffered owing to hypothyroidism. Contrarily to these data, PTU did not cause any alteration in circulating levels of TNF- α in human [10] nor in rats [11].

Table 2. Serum sitokin seviyeleri (mean±SEM, n=5)**Table 2.** Serum cytokine levels (mean±SEM, n=5)

Group	IL-1β (pg/mL)	IL-6 (pg/mL)	IL-10 (pg/mL)	TNF-α (pg/mL)
Control	28.02±2.39	30.20±2.35	36.51±1.63	39.61±2.09 b
Tx	39.05±5.49	36.04±3.73	38.93±1.85	46.23±1.01 a

a, b Different letters in the same column refers the differences between the groups (P<0.05)

In this regard, we have investigated the serum levels of IL-1, -6, -10 and TNF-α in thyroidectomized rats. In the present study, serum TSH levels approximately ~11 fold higher (P<0.05) and FT3 levels decreased (P<0.05) in the thyroidectomized group compared with control. The obtained data shown that hypothyroidism have been well established after thyroidectomy operation.

IL-1 inhibits TSH-induced TPO gene expression directly, thus IL-1 as a local inhibitor of THs synthesis. Additionally, IL-6 can inhibits TSH-induced TPO mRNA transcription in a dose-dependent manner [1].

Pro-inflammatory or anti-inflammatory cytokines which produced by Th1 (IL-1β, TNF-α) and Th2 (IL-6, IL-10) cells have crucial role in protecting against the autoimmune thyroid disease [4,12]. Furthermore, now it is well known that IL-1, IL-6 and TNF-α could play central role in the pathogenesis of non thyroidal illness (NTI) syndrome by acting on hypothalamus pituitary-thyroid axis [13]. IL-1, IL-6 and TNF-α are effect as suppressor mediators on thyroid hormones during NTI [14]. Also, Bartalena et al. [15] tested that hypothesis in rats with induced hypothyroidism by administration of the methimazole throughout 3 weeks and they concluded that 48 hours after a single dose recombinant human IL-6 injection could reduce serum T3, T4 and TSH concentrations due to central effects of the cytokine.

From another aspect, trauma and wound healing could lead to significant elevation in pro-and anti-inflammatory cytokines [16,17]. Especially IL-1β and TNF-α, IL-6 and C-reactive protein, which is a predictor for acute-phase response and IL-10 have been used in biomarkers for monitoring traumatic inflammation and recovery period in clinically both human and veterinary medicine [18-20]. Ozcinar et al. [21] reported the pro-inflammatory cytokines IL-1β and TNF-α levels were higher after thyroid surgery in human. However, the sample size restricted to interpret of the obtained data in the present study. Slight increased levels of pro-inflammatory cytokines IL-1, IL-6 due to their stimulator effects on TNF-α production raised the level significantly, meanwhile slight elevation on the level of anti-inflammatory cytokine IL-10 might explain as a consequence of the thyroidectomy operation in accordance with previous studies [19,21].

In conclusion, surgical thyroidectomy intervention may cause of alteration in levels of cytokines on post-operative period. Therefore, whilst used in hypothyroid animals as

a model in experimental studies, these data have to take into consideration.

REFERENCES

1. **Ajjan RA, Watson PF, Weetman AP:** Cytokines and thyroid function. *Adv Neuroimmunol*, 6, 359-386, 1996. DOI: 10.1016/S0960-5428(97)00027-7
2. **Aggarwal BB, Gupta SC, Kim JH:** Historical perspectives on tumor necrosis factor and its superfamily: 25 years later, a golden journey. *Blood*, 119, 651-665, 2012. DOI: 10.1182/blood-2011-04-325225
3. **Akdis M, Burgler S, Cramer R, Eiwegger T, Fujita H, Gomez E, Klunker S, Meyer N, O'Mahony L, Palomares O, Rhyner C, Ouaked N, Schaffartzik A, Van De Veen W, Zeller S, Zimmermann M, Akdis CA:** Interleukins, from 1 to 37, and interferon-gamma: receptors, functions, and roles in diseases. *J Allergy Clin Immunol*, 127, 701-721 e701-770, 2011. DOI: 10.1016/j.jaci.2010.11.050
4. **Mikos H, Mikos M, Obara-Moszyńska M, Niedziela M:** The role of the immune system and cytokines involved in the pathogenesis of autoimmune thyroid disease (AITD). *Endokrynol Pol*, 65, 150-155, 2014. DOI: 10.5603/EP.2014.0021
5. **Koibuchi N:** Animal models to study thyroid hormone action in cerebellum. *Cerebellum*, 8, 89-97, 2009. DOI: 10.1007/s12311-008-0089-x
6. **Volpe R:** The immunomodulatory effects of anti-thyroid drugs are mediated via actions on thyroid cells, affecting thyrocyte-immunocyte signalling: a review. *Curr Pharm Des*, 7, 451-460, 2001. DOI: 10.2174/1381612013397898
7. **Shirin H, Dotan I, Papa M, Maaravi Y, Aeed H, Zaidel L, Matas Z, Bruck R, Moss SF, Halpern Z, Oren R:** Inhibition of concanavalin A-induced acute T cell dependent hepatic damage in mice by hypothyroidism. *Liver*, 19, 206-211, 1999. DOI: 10.1111/j.1478-3231.1999.tb00037.x
8. **Bruck R, Oren R, Shirin H, Aeed H, Papa M, Matas Z, Zaidel L, Avni Y, Halpern Z:** Hypothyroidism minimizes liver damage and improves survival in rats with thioacetamide induced fulminant hepatic failure. *Hepatology*, 27, 1013-1020, 1998. DOI: 10.1002/hep.510270417
9. **Kızıltunc A, Basoglu M, Avci B, Capoglu I:** Serum IL-6 and TNF-α in patients with thyroid disorders. *Turk J Med Sci*, 29 (1): 25-30, 1999.
10. **Elias AN, Nanda VS, Pandian R:** Serum TNF-alpha in psoriasis after treatment with propylthiouracil, an antithyroid thioureydene. *BMC Dermatol*, 30, 4, 2004. DOI: 10.1186/1471-5945-4-4
11. **Sener G, Sehirli O, Velioglu-Ogunc A, Ercan F, Erkanli G, Gedik N, Yegen BC:** Propylthiouracil (PTU)-induced hypothyroidism alleviates burn-induced multiple organ injury. *Burns*, 32, 728-736, 2006. DOI: 10.1016/j.burns.2006.01.002
12. **Sahna KC, Risvanli A:** Th1/Th2 cytokine balance and SOCS3 levels of female offspring born from rats with gestational diabetes mellitus. *Kafkas Univ Vet Fak Derg*, 21, 837-840, 2015. DOI: 10.9775/kvfd.2015.13723
13. **de Vries EM, Fliers E, Boelen A:** The molecular basis of the non-thyroidal illness syndrome. *J Endocrinol*, 225(3):R67-81, 2015. DOI: 10.1530/JOE-15-0133
14. **van Haasteren GA, van der Meer MJ, Hermus AR, Linkels E, Klootwijk W, Kaptein E, van Toor H, Sweep CG, Visser TJ, de Greef WJ:** Different effects of continuous infusion of interleukin-1 and interleukin-6 on the hypothalamic-hypophysial-thyroid axis. *Endocrinology*, 135, 1336-1345, 1994. DOI: 10.1210/endo.135.4.7925094

- 15. Bartalena L, Grasso L, Brogioni S, Martino E:** Interleukin 6 effects on the pituitary-thyroid axis in the rat. *Eur J Endocrinol*, 131, 302-306, 1994. DOI: 10.1530/eje.0.1310302
- 16. Uluisik D, Keskin E:** The effects of ginseng and echinacea on some plasma cytokine levels in rats. *Kafkas Univ Vet Fak Derg*, 18, 65-68, 2012. DOI: 10.9775/kvfd.2011.4814
- 17. Hismiogullari SE, Hismiogullari AA, Yavuz MT, Yavuz O, Yaman I, Seyrek K, Hayirli A, Rahman K:** The protective effect of resveratrol in experimentally induced non-sterile clean wound inflammation in rats. *Kafkas Univ Vet Fak Derg*, 19 (Suppl.-A): A1-A5, 2013. DOI: 10.9775/kvfd.2012.6989
- 18. Schijns VECJ, Horzinek MC:** Cytokines in veterinary medicine. Wallingford: *CAB International*, 1997.
- 19. Reikeras O, Borgen P, Reseland JE, Lyngstadaas SP:** Changes in serum cytokines in response to musculoskeletal surgical trauma. *BMC Res Notes*, 7, 128, 2014. DOI: 10.1186/1756-0500-7-128
- 20. Ok M, Er C, Yildiz R, Col R, Aydogdu U, Sen İ, Guzelbektes H:** Evaluation of acute phase proteins, some cytokines and hemostatic parameters in dogs with sepsis. *Kafkas Univ Vet Fak Derg*, 21, 761-766, 2015. DOI: 10.9775/kvfd.2015.13418
- 21. Ozcinar B, Aksakal N, Yanar F, Agcaoglu O, Peker KD, Turkoglu U, Mercan S, Ozarmagan S, Erbil Y:** Increased interleukin 1 and tumor necrosis factor alpha levels after thyroid surgery. *Ulus Cerrahi Derg*, 30, 80-84, 2014. DOI: 10.5152/UCD.2014.2484