Available online at www.medicinescience.org

ORIGINAL ARTICLE

Medicine Science International Medical Journal

Medicine Science 2020;393-8

Investigation of renal and testicular pro-inflammatory cytokine levels in experimental hyperthyroidism: Effects of selenium supplementation

Nurten Bahtiyar, Aysun Yoldas, Selmin Toplan

Istanbul University Cerrahpasa, Cerrahpasa Medical Faculty, Department of Biophysics, Istanbul, Turkey

Received 06 January 2020; Accepted 23 March 2020
Available online 16.05.2020 with doi: 10.5455/medscience.2020.09.9224

Abstract

Cytokines play important roles in diseases. Pro-inflammatory cytokines may responsible for thyroid dysfunction. Selenium (Se) regulates pro-inflammatory status in the body. Kidney and testicular functions change in hyperthyroidism. The aim of this study was to investigate the effects of Se supplementation on pro-inflammatory cytokines in renal and testicular tissue in hyperthyroid rats. This study was carried out with 6 experimental groups (G). G-1 consisted of controls receiving standard rat fodder. Other experimental groups were fed with; G-2: 0.5 mg/kg Na_2SeO_3; G-3: 1 mg/kg Na_2SeO_3; G-4: 4 mg/kg L-thyroxine; G-5: 0.5 mg/kg Na_2SeO_3 and 4 mg/kg L-thyroxine; G-6: 1 mg/kg Na_2SeO_3 and 4 mg/kg L-thyroxine added standard fodder. Interleukin (IL)-1β, IL-6, IL-18, and TNF-α levels were studied in renal and testicular tissues by ELISA. All cytokines levels of kidney samples were measured in tissue homogenates. Kidney values of IL-1β, IL-18, IL-6 and TNF-α were increased in G-4 compared to G-1 (p=0.024, p=0.010, p=0.003, and p=0.011, respectively). Also, these cytokine levels were decreased in G-6 compared to G-4 (p=0.042, p=0.048, p=0.009, and p=0.021, respectively). The IL-1β, IL-18, IL-6 and TNF-α values of G-4 were higher than G-1 in testes samples (p=0.027, p=0.010, p=0.008, and p=0.009, respectively). In addition, G-6 levels of IL-18 and TNF-α were lower than G-4 (p=0.047, and p=0.032 respectively), but IL-1β and IL-6 changes were not significant. As a result, our findings showed that in hyperthyroidism IL-1β, IL-18, IL-6, and TNF-α levels increased in kidney and testis tissues. Also, supplementation of 1 mg/kg Na_2SeO_3 was more effective for decreasing levels of these pro-inflammatory cytokine levels in the hyperthyroid group than supplementation of 0.5 mg/kg Na_2SeO_3.

Keywords: Hyperthyroidism, pro-inflammatory cytokines, kidney, testis, selenium

Introduction

Thyroid diseases are emerging as one of the most common endocrine disorders. Hyperthyroidism is a disorder in which excess thyroid hormone is synthesized and secreted by the thyroid gland. Differences in thyroid functions affect almost all physiological systems [1, 2].

Cytokines are small molecules, which are synthesized by many different cell types, played important roles in health and disease [3]. These molecules have pleiotropic and generally overlapping effects and are important for autoimmune response. Interleukin (IL)-1β, IL-6, IL-8, and Tumor necrosis factor-α (TNF-α) are pro-inflammatory cytokines, and these molecules affect many systems [4].

***Coresponding Author:** Nurten Bahtiyar, Istanbul University-Cerrahpasa, Cerrahpasa Medical Faculty, Department of Biophysics, Istanbul, Turkey, E-mail: nurtenbahtiyar@hotmail.com

The cytokines may act on the cells that secrete them (autocrine action), on nearby cells (paracrine action), or in some instances on distant cells (endocrine action) [5]. They are produced by a large number of cells, including thyroid follicular cells [4]. The relationship between thyroid hormones and immune cells is complex. Triiodothyronine (T3) and thyroxine (T4) levels may modulate immune responses through both genomic and non-genomic mechanisms [6]. It is reported that thyroid hormones increase the production of inflammatory cytokines [7].

Thyroid hormones influence kidney structure, renal hemodynamics, and kidney development. The changes in renal function occur in both hypothyroidism and hyperthyroidism. It has been reported that thyroid dysfunction is associated with the development of immune-mediated glomerular damage, and thyroid hormones are changed in individuals with kidney disease [1].

Hyperthyroidism also affects the reproductive system. Infertility, gynecomastia, and sexual impotence may occur in men with hyperthyroidism [8, 9]. Testicular functions have been reported to

be suppressed by increased systemic levels of some cytokines [10].

Selenium (Se) is a trace element, which is found in the selenocysteine form in selenoproteins. Se acts as a regulator of thyroid hormone metabolism. It is reported that Se may be effective in the course of many inflammatory diseases. Also, there is strong evidence that inflammation may vary depending on the presence of Se. It was declared that decreased serum Se levels were seen in acute and chronic inflammatory conditions [11].

Hyperthyroidism was easily achieved in rodents by continuous treatment of L-thyroxine, a synthetic form of thyroid hormone [12]. Administration of L-thyroxine enhances the concentration of serum thyroid hormones and indicating the induction of the hyperthyroid state [13].

In literature, the cytokine changes in hyperthyroidism were mostly examined in the circulation, but these studies reported conflicting results. In some studies have reported high levels of circulatins pro-inflammatory cytokines in hyperthyroidism, whereas others have not found significant changes [14-18]. Also, the kidney and testicular dysfunction are developed in some patients with hyperthyroidism. [1, 19-21]. Using the knowledge of the changes circulating levels of pro-inflammatory cytokines in hyperthyroidism, and the role of pro-inflammatory cytokines in the development of kidney and testicular dysfunctions, in this study, it is investigated pro-inflammatory cytokines levels in renal and testicular tissues in hyperthyroid rats. In addition, by considering the anti-inflammatory properties of Se, we aimed to evaluate the effect of Se supplementation on these markers in hyperthyroidism.

Materials and Methods

Study design

The experimental study was carried out with 48 Wistar-Albino male rats. Their body weight ranged from 235 -265 g. All animals were kept under the same environmental conditions, i.e. at a room temperature of 25˚C, with an artificial light cycle (lights: 08:00–20:00 h), and were left for one week for adaptation. Hyperthyroidism was induced by continuous treatment of L-thyroxine [12]. The animals were divided into 6 experimental groups (G) as G-1, G-2, G-3, G-4, G-5 and G-6, each group consisting of 8 animals. G-1 consisted of controls receiving standard rat fodder. Other experimental groups were fed with; G-2: 0.5 mg/kg Na_2SeO_3; G-3: 1 mg/kg Na_2SeO_3; G-4: 4 mg/kg L-thyroxine; G-5: 0.5 mg/kg Na_2SeO_3 and 4 mg/kg L-thyroxine; G-6: 1 mg/kg Na_2SeO_3 and 4 mg/kg L-thyroxine added standard fodder during 30 days [22-24]. All animals drank tap water. At the end of the 30-day experimental period, the blood samples were collected via cardiac puncture. Blood samples were taken in tubes containing gel. Serum samples were removed from blood samples after centrifugation at 3000 x g for 20 min at 4°C. Serum samples were stored in polyethylene eppendorf tubes at -80 ˚C until analysis. Our protocol and methods were approved by the Animal Care and Use Committee of Laboratory Animal Service of Istanbul University, Istanbul, Turkey (Number: 2013/37).

Hormone Measurements

Serum thyroid stimulating hormone (TSH), free T4 (fT4), and free T3 (fT3) levels were measured by ELISA kits (Sunred biological technology, Shangai, China). All the procedures were carried out following the manufacturer's instructions.

Se Measurement in Serum Samples

Serum Se levels were measured by inductively coupled plasma optical emission spectrophotometer (ICP-OES, Thermo iCAP 6000, Cambridge, UK). Serum samples were diluted 1:10 with 0.3% HNO3 (Merck, Darmstadt, Germany) for analysis. Calibration standards were prepared using stock solution (Chem-Lab, Belgium) at concentrations of 1000 mg/L. Elemental solutions of 0.25, 0.50, 1.00, 25.00 and 50.00 µg/dl concentrations were prepared by using stock solution and distilled water (Millipore, Bedford, MA, ABD) containing 0.3% HNO3. The recovery rate of Se standard solutions was 99.82 %. Se element levels were determined by using 196.026 nm wavelength. Results were given as µg/dl.

Preparation of Tissue Homogenates

Kidney and testis tissues from all rats were excised, rinsed with ice-cold saline and homogenized in 100 mMTris–HCl buffer (pH 7.4) using homogenizer. The lysate was then cold-centrifuged at 12 000 x g for 30 min at 4 °C. Supernatants were collected and stored at -80°C until analyzed. Protein concentrations of supernatants were determined by Lowry's method [25].

Measurement of Cytokine Levels

The levels of IL-1β, IL-6, IL-18, and TNF-α in kidney and testis tissue samples were quantified according to the manufacturer's instructions and guidelines using an ELISA kit specific for the rat cytokines (Sunred biological technology, Shangai, China). Cytokine levels were determined in the supernatants of tissue homogenates, and the concentrations were expressed as pg/mg protein.

Statistical analysis

After checking for the normality assumptions with the Shapiro-Wilk normality test, statistical analysis was performed using one way ANOVA and Bonferroni post hoc test. Data are presented as mean ± the standard deviation (SD). $p<0.05$ was considered to indicate a statistically significant difference. All calculations were performed using GraphPad Prism version 5.00 for Windows (GraphPad Software, Inc., La Jolla, CA, USA).

Results

Thyroid Function Markers

Serum values of fT3 were increased in G-4 (1.98±0.35) compared to G-1 (1.51±0.29; p=0.046). Also, fT4 levels were increased in G-4 (11.76±2.33) compared to G-1 (8.62±1.95; p=0.004).

But, TSH values of G-4 (0.65±0.13) were higher than G-1 (0.79±0.13; p =0.045) (Table 1).

Serum Se Levels

The Se levels of G-4 (20.09±1.60) were lower than G-1 (23.62±1.44

p=0.049). Se levels of G-5 (23.43±3.29) were lower than G-2 (28.79±2.31; p =0.004). The levels of G-6 (28.59±2.38) were higher than G-5 (23.43±3.29; p=0.005), and G-4 (20.09±1.60; p<0.001) (Table 1).

Cytokines Levels of Kidney Tissues

All cytokines levels of kidney samples were measured in tissue homogenates. Kidney values of IL-1β were increased in G-4 (56.98±5.48) compared to G-1 (46.01±3.59; p=0.024). Also, IL-1β levels were decreased in G-6 (46.75±5.85) compared to G-4 (56.98±5.48; p=0.042) (Table 2).

Kidney values of IL-18 were higher in G-4 (13.99±1.48) compared to G-1(10.54±1.81; p=0.010), and lower in G-6 (11.05±2.29) compared to G-4 (13.99±1.48; p=0.048) (Table 2).

IL-6 levels increased in G-4 (21.91±2.73) compared to G-1 (16.10±2.08; p=0.003). The values of G-5 (19.81±2.03b) were higher than G-2 (15.00±1.46; p=0.029). In addition, IL-6 values of G-6 (16.25±5.09) were lower than G-4 (21.91±2.73; p=0.009) (Table 2).

TNF-α values increased in G-4 (14.91±1.30) compared to G-1 (10.89±0.99; p=0.011). In addition, TNF-α values of G-6 (11.61±4.34) were lower than G-4 (14.91±1.30; p=0.021) (Table 2).

Cytokines Levels of Testis Tissues

The cytokines levels were measured in homogenate samples. Testis IL-1β values were increased in G-4 (45.80±2.52) compared to G-1 (29.74±1.76; p=0.027). Also, IL-1β levels were increased in G-5 (37.29±4.66) compared to G-2 (45.80±2.52; p=0.023) (Table 3).

The testis values of IL-18 were increased in G-4 (10.12±0.89) compared to G-1(7.68±2.04; p=0.010). IL-18 levels were increased higher in G-5 (9.02±0.89) compared to G-2 (6.07±1.13; p=0.001), and lower in G-6 (8.34±1.15) compared to G-4 (10.12±0.89; p=0.047) (Table 3).

IL-6 levels increased in G-4 (33.04±4.73) compared to G-1 (21.05±6.61; p=0.008) (Table 3).

TNF-α values increased in G-4 (34.32±5.83) compared to G-1 (24.53±4.88; p=0.009). In addition, TNF-α values of G-6 (25.67±6.05) were lower than G-4 (34.32±5.83; p=0.032) (Table 3).

Table 1. Serum thyroid function parameters and selenium levels in all studied groups

	G-1	G-2	G-3	G-4	G-5	G-6
fT3(ng/ml)	1.51±0.29	1.64±0.36	1.70±0.29	1.98±0.35a*	1.89±0.36	1.69±0.26
fT4(pmol/l)	8.62±1.95	9.94±1.21	9.42±1.61	11.76±2.33a**	10.1±1.1	9.45±0.65
TSH(mlU/)	0.79±0.13	0.69±0.09	0.71±0.07	0.65±0.13a*	0.68±0.13	0.80±0.08
Se (µg/dL)	23.62±1.44	28.79±2.31	32.60±1.97	20.09±1.60a*	23.43±3.29b**	28.59±2.38c***

Values are presented as the mean ± the standard deviation. G-1 consisted of controls receiving standard rat fodder. Other experimental groups were fed with; G-2: 0.5 mg/kg Na_2SeO_3; G-3: 1 mg/kg Na_2SeO_3; G-4: 4 mg/kg L-thyroxine; G-5: 0.5 mg/kg Na_2SeO_3 and 4 mg/kg L-thyroxine; G-6: 1 mg/kg Na_2SeO_3 and 4 mg/kg L-thyroxine added standard fodder during 30 days, aG-1 vs. Group-4; bG-2 vs. G-5, cG-4 vs. G-6, *p<0.05, **p<0.01, ***p<0.001.

Table 2. Kidney pro-inflammatory cytokines levels of all studied groups

	G-1	G-2	G-3	G-4	G-5	G-6
IL-1β (pg/mg protein)	46.01±3.59	44.70±7.33	39.36±5.68	56.98±5.48a*	47.52±9.56	46.75±5.85c*
IL-18 (pg/mg protein)	10.54±1.81	9.80±1.50	9.34±1.63	13.99±1.48a*	11.73±2.36	11.05±2.29c*
IL-6 (pg/mg protein)	16.10±2.08	15.00±1.46	14.07±3.82	21.91±2.73a**	19.81±2.03b*	16.25±5.09c**
TNF-α (pg/mg protein)	10.89±0.99	10.20±1.73	9.39±2.04	14.91±1.30a*	13.20±3.25	11.61±4.34c*

Values are presented as the mean ± the standard deviation. G-1 consisted of controls receiving standard rat fodder. Other experimental groups were fed with; G-2: 0.5 mg/kg Na_2SeO_3; G-3: 1 mg/kg Na_2SeO_3; G-4: 4 mg/kg L-thyroxine; G-5: 0.5 mg/kg Na_2SeO_3 and 4 mg/kg L-thyroxine; G-6: 1 mg/kg Na_2SeO_3 and 4 mg/kg L-thyroxine added standard fodder during 30 days. aG-1 vs. Group-4, bG-2 vs. G-5, cG-4 vs. G-6. *p<0.05, **p<0.01.

Table 3. Testis pro-inflammatory cytokines levels of all studied groups

	G-1	G-2	G-3	G-4	G-5	G-6
IL-1β (pg/mg protein)	29.74±1.76	26.41±2.42	25.48±3.66	45.80±2.52a*	37.29±4.66b*	32.24±7.42
IL-18 (pg/mg protein)	7.68±2.04	6.07±1.13	5.49±1.46	10.12±.0.89a*	9.02±0.89b***	8.34±1.15c*
IL-6 (pg/mg protein)	21.05±6.61	20.53±3.29	19.51±3.31	33.04±4.73a**	25.73±6.05	22.84±3.27
TNF-α (pg/mg protein)	24.53±4.88	23.95±4.57	21.43±6.42	34.32±5.83a**	29.72±3.35	25.67±6.05c*

Values are presented as the mean ± the standard deviation. G-1 consisted of controls receiving standard rat fodder. Other experimental groups were fed with; G-2: 0.5 mg/kg Na_2SeO_3; G-3: 1 mg/kg Na_2SeO_3; G-4: 4 mg/kg L-thyroxine; G-5: 0.5 mg/kg Na_2SeO_3 and 4 mg/kg L-thyroxine; G-6: 1 mg/kg Na_2SeO_3 and 4 mg/kg L-thyroxine added standard fodder during 30 days. aG-1 vs. Group-4, bG-2 vs. G-5, cG-4 vs. G-6. *p<0.05, **p<0.01.

Discussion

Cytokines are soluble proteins that exhibit their actions in the local environment or systemically to alter and regulate immunological and inflammatory reactions [26]. They are important molecules that affect functions of tissues [4]. Some hyperthyroid patients also have kidney and testicular dysfunction. Increased pro-inflammatory cytokine levels may cause tissue damage. In this study, we investigated pro-inflammatory cytokine levels in kidney and testicle tissue in hyperthyroid rats. We also examined the effect of Se supplementation on these markers.

Our findings were showed that the kidney tissue levels of IL-1β, IL-18, IL-6, and TNF-α cytokines are increased in the hyperthyroid group. According to our results, the IL-1β, IL-18, IL-6, and TNF-α were increased by 23.84%, 32.72%, 36.08%, and 36.91% respectively in the hyperthyroid group in comparison with the control.

The studies examining the relationship between thyroid and kidney function concentrate on cytokines measured in the circulation [27-29]. Also, the information on the effects of the cytokines was obtained by in vitro incubating the cells with cytokines. However, this can be misleading for two reasons. First, cytokines do not act as a single agent acting on a particular cell type. Second, most of the effects of cytokines are local, not systemic [26, 30].

A study examining the effect of pro-inflammatory markers on renal function reported that higher CRP and soluble TNF receptor 2 are associated with faster renal function loss rates in chronic renal failure [31]. It has been demonstrated a significant association between plasma TNF-α levels and progressive loss of renal function in chronic renal failure [32]. Also, it has been reported that thyroid hormones secreted by the thyroid gland may affect the kidney via the immune system [21].

Thyroid hormones can affect glomerular and tubular functions through prerenal and intrarenal effects. The formation and accumulation of immune complexes in the glomerular is the key point at the onset of nephropathy [33]. Patients with thyroid disorders are at risk for immune-mediated glomerular diseases [1]. Our data is compatible with this information, and the increase of pro-inflammatory markers may indicate that the inflammatory process occurred in the kidney tissue due to hyperthyroidism.

Low serum Se levels are a frequent finding in patients with acute kidney injury or chronic kidney disease. But, the relationship between the low Se levels and the comorbidities associated with renal disease has not been extensively evaluated. It was suggested that low Se levels may contribute to immune dysfunction, increasing the risk of death from infectious disease in hemodialysis patients. Also, some studies have reported that Se status and immune function improve after Se supplementation in renal patients [34]. However, to our knowledge, there was no study investigating the levels of pro-inflammatory markers due to antioxidant supplementation in the kidney tissues in hyperthyroidism. Our findings were showed that the levels of IL-1β, IL-18, IL-6, and TNF-α cytokines decreased in the two Se supplemented hyperthyroid groups. But all pro-inflammatory cytokines in 0.5mg/kg Se supplemented hyperthyroid group was not decreased significantly when compared to the hyperthyroid group. The IL-1β, IL-18, IL-6, and TNF-α levels were decreased in the 1 mg/kg Se supplemented hyperthyroid group significantly compared to the hyperthyroid group ($p=0.042$, $p=0.048$, $p=0.009$, and $p=0.021$, respectively). These data showed that Se administration was beneficial in decreasing renal cytokine levels.

Also in this study, the levels of IL-1β, IL-18, IL-6, and TNF-α cytokines in testis tissue were investigated in experimentally induced hyperthyroid rats. Our findings were showed that the testis levels of IL-1β, IL-6, IL-18, and TNF-α levels were increased by 53.99%, 31.77%, 56.95%, and 39.91% respectively in the hyperthyroid group in comparison to control. But, in literature, we did not see the study investigating pro-inflammatory markers in testicular tissue in hyperthyroidism.

Cytokines normally function as paracrine regulators of testicular spermatogenesis and steroidogenesis. In the case of inflammation, complex and multiple interactions between immune and germ cells lead to changes in spermatogenesis. The complexity of these cell interactions is due to the fact that sertoli and leydig cells also produce pro- and anti-inflammatory cytokines and chemokines. Despite the two-way immunoregulatory function of cytokines, they show upregulation of interleukin IL-1α, IL-1β, IL-6, and TNF-α in acute testicular inflammation, and have adverse effects on germ cells. In the event of chronic damage to the testis, the interaction of germ and somatic testicular immune cells can disrupt the spermatogenesis process [35]. To our findings, the increase of these markers in testicular tissue may suggest the relationship between testicular dysfunction and infertility in hyperthyroidism.

The element Se is very important for testicular tissue. This element maintains normal spermatogenesis, and is essential for male fertility. In the event of deficiency of Se, the level of this element in the gonads by the regulatory mechanisms try to maintain and in the case of Se supplementation, Se is primarily given to the testes then other tissues [36]. Se, also has some immunomodulatory roles that are evident by the inhibition of inflammatory cytokine activation in sertoli cells [37]. In this study, it is evaluated the levels of pro-inflammatory markers due to Se supplementation in the testis tissues. Our findings were showed that the levels of IL-1β, IL-18, IL-6, and TNF-α cytokines decreased in our two Se supplemented hyperthyroid groups. But all pro-inflammatory cytokines in 0.5mg/kg Se supplemented hyperthyroid group was not decreased significantly when compared to the hyperthyroid group. While the IL-18 and TNF-α levels were decreased significantly in the 1 mg/kg Se supplemented hyperthyroid group in comparison to the hyperthyroid group ($p=0.047$, $p=0.032$, respectively), the IL-1β and IL-6 levels were not decreased significantly. These data showed that Se supplementation reduced cytokines in testicular tissues of hyperthyroid rats. These findings suggest the idea that Se supplementation may be used to prevent infertility.

But our study has several limitations. It was examined a limited number of pro-inflammatory cytokines in this study. Also, we did not evaluate the time-dependent changes in cytokine levels. It could be useful to examine also the levels of anti-inflammatory markers at the same time, to be informed about the pro-inflammatory and anti-inflammatory status. Also, it could be beneficial to determine the relationship between cytokines levels in the thyroid and selected tissues.

Conclusion

In conclusion, our findings suggested that pro-inflammatory cytokine levels were increased in renal and testis tissues of hyperthyroid rats, Se supplementation 0.5 mg/kg in the diet reduced these cytokine levels, but 1 mg/kg Se supplementation was more effective in the reduction of cytokines. These findings suggest that appropriate doses of Se supplementation may be beneficial in patients with renal and testicular dysfunction due to hyperthyroidism.

Conflict of interests
The authors declare that they have no conflict of interest and any financial disclosures.

Financial Disclosure
The present study was supported by the Research Fund of the Istanbul University (Project ID: 1766).

Ethical approval
The study was approved by Board of Ethics in Animal Experiments of Istanbul University

References

1. Mariani LH, Berns JS. The renal manifestations of thyroid disease. J Am Soc Nephrol. 2012;23:22-6.
2. Kahaly GJ, Bartalena L, Hegedüs L, et al. 2018 European Thyroid Association Guideline for the Management of Graves' Hyperthyroidism. Eur Thyroid J. 2018;7:167-86.
3. Gianoukakis AG, Khadavi N, Smith TJ. Cytokines, Graves' disease, and thyroid-associated ophthalmopathy. Thyroid. 2008; 18:953-8.
4. Senturk T, Kozaci LD, Kok F, et al. Proinflammatory cytokine levels in hyperthyroidism. Clin Invest Med. 2003;26:58-63.
5. Zhang J-M, An J. Cytokines, Inflammation and Pain. Int Anesthesiol Clin. 2007;45:27–37.
6. De Vito P, Incerpi S, Pedersen JZ, et al Thyroid hormones as modulators of immune activities at the cellular level. Thyroid. 2011;21:879-90.
7. Rozing MP, Westendorp RG, Maier AB, et al. Serum triiodothyronine levels and inflammatory cytokine production capacity. Age (Dordr). 2012;34:195-201.
8. Hudson RW, Edwards AL. Testicular function in hyperthyroidism. J Androl. 1992;13:117-24.
9. Wajner SM, Wagner MS, Maia AL. Clinical implications of altered thyroid status in male testicular function. Arq Bras Endocrinol Metabol. 2009;53:976-82.
10. Handelsman DJ. Testicular dysfunction in systemic disease. Endocrinol Metab Clin North Am. 1994;23:839-56.
11. Duntas LH. Selenium and inflammation: underlying anti-inflammatory mechanisms. Horm Metab Res. 2009;41:443-7.
12. Kim S-M, Kim S-C, Chung I-K, et al. Antioxidant and Protective Effects of Bupleurum falcatum on the l-Thyroxine-Induced Hyperthyroidism in Rats. Evid Based Complement Alternat Med. 2012;2012:578497.
13. Panda S, Kar A. Amelioration of L-thyroxine-induced hyperthyroidism by coumarin (1,2-benzopyrone) in female rats. Clin Experimental Pharmacol Physiol. 2007;34:1217–9.
14. Weetman AP, Bright-Thomas R, Freeman M. Regulation of interleukin-6 release by human thyrocytes. J Endocrinol. 1990;127:357-61.
15. Yamada T, Sato A, Aizawa T. Dissociation between serum interleukin-6 rise and other parameters of disease activity in subacute thyroiditis during treatment with corticosteroid. J Clin Endocrinol Metab. 1996;81:577-9.
16. Bartalena L, Brogioni S, Grasso L, Martino E.Increased serum interleukin-6 concentration in patients with subacute thyroiditis: relationship with concomitant changes in serum T4-binding globulin concentration. J Endocrinol Invest. 1993;16:213-8.
17. Szabo-Fresnais N, Blondeau JP, Pomérance M. Activation of the cAMP pathway synergistically increases IL-1-induced IL-6 gene expression in FRTL-5 thyroid cells: involvement of AP-1 transcription factors. Mol Cell Endocrinol. 2008;284:28-37.
18. Zheng RQ, Abney ER, Chu CQ, et al. Detection of in vivo production of tumour necrosis factor-alpha by human thyroid epithelial cells. Immunology. 1992;75:456–62.
19. Lotti F, Maseroli E, Fralassi N, et al. Is thyroid hormones evaluation of clinical value in the work-up of males of infertile couples? Hum Reprod. 2016;31:518-29.
20. Abalovich M, Levalle O, Hermes R, et al. Hypothalamic pituitary-testicular axis and seminal parameters in hyperthyroid males. Thyroid. 1999;9:857–63.
21. Abozenah H, Shoeb S, Sabry A, et al. Relation between thyroid hormone concentration and serum levels of interleukin-6 and interleukin-10 in patients with nonthyroidal illness including chronic kidney disease. Iran J Kidney Dis. 2008;2:16-23.
22. Amara IB, Troudi A, Garoui E et al. Protective effects of selenium on methimazole nephrotoxicity in adult rats and their offspring. Experimental Toxicol Pathol. 2011;63:553-561.
23. Erkekoglu P, Zeybek ND, Giray BK et al. The effects of di (2-ethylhexyl) phthalate on rat liver in relation to selenium status. Int J Experimental Pathol. 2014;95:64-77.
24. Ozdemir S, Yucel R, Dariyerli N et al. The effects of experimental hyperthyroidism on hemorheology and plasma fibrinogen concentration. Endocrine. 2006;30:203-5.
25. Lowry OH, Rosebrough NJ, Farr AL, et al. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951;193:265-75.
26. Ortega L, Fornoni A. Role of cytokines in the pathogenesis of acute and chronic kidney disease, glomerulonephritis, and end-stage kidney disease. Int J Interferon, Cytokine Mediator Res. 2010;2:49-62.
27. Karzakova LM, Avtonomova OI, Kudryashov SI, et al. The role of circulating cytokines and thyroid hormones in the development of the nephrotic variant of glomerulonephritis. Patol Fiziol Eksp Ter. 2016;60:76-82.
28. Zoccali C, Tripepi G, Cutrupi S, et al. Low triiodothyronine: a new facet of inflammation in end-stage renal disease. J Am Soc Nephrol. 2005;16:2789-95.
29. Carrero JJ, Qureshi AR, Axelsson J, et al. Clinical and biochemical implications of low thyroid hormone levels (total and free forms) in euthyroid patients with chronic kidney disease. J Intern Med. 2007;262:690-701.
30. Schindler R, Krautzig S, Lufft V, et al. Induction of interleukin-1 and interleukin-1 receptor antagonist during contaminated in-vitro dialysis with whole blood. Nephrol Dial Transplant. 1996;11:101-8.
31. Tonelli M, Sacks F, Pfeffer M, et al. Cholesterol and Recurrent Events (CARE) Trial Investigators. Biomarkers of inflammation and progression of chronic kidney disease. Kidney Int. 2005;68:237-45.
32. Amdur RL, Feldman HI, Gupta J, et al. Inflammation and Progression of CKD: The CRIC Study. Clin J Am Soc Nephrol. 2016;11:1546-56.
33. Hao Y, Wu X, Huang X, et al. Proteinuria Associated with Thyroid Disorders-Cases Report and Literature Review. Med Case Rep. 2017;3:35.

34. Iglesias P, Selgas R, Romero S, et al. Selenium and kidney disease. J Nephrol. 2013;26:266-72.

35. Guazzone VA, Jacobo P, Theas MS, et al. Cytokines and chemokines in testicular inflammation: A brief review. Microsc Res Tech. 2009;72:620-8.

36. Boitani C1, Puglisi R. Selenium, a key element in spermatogenesis and male fertility. Adv Exp Med Biol. 2008;636:65-73.

37. Qazi IH, Angel C, Yang H, et al. Role of Selenium and Selenoproteins in Male Reproductive Function: A Review of Past and Present Evidences. Antioxidants (Basel). 2019;2:8: