WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 7.421
Volume 7, Issue 4, 64-76 Research Article ISSN 2278 – 4357

SERUM INTERLEUKIN-6 AND GASTRIC MUCOSA EXPRESSION OF CD3 AND CD20 IN DYSTHYROID RATS

Ajayi Ayodeji Folorunsho[1]*, Adelakun Ayodele Ademola[2], Abidoye Oluwafemi Emmanuel[1], Adeleye Gbenga Sunday[3] and Madukwe Jonathan[4]

[1]Department of Physiology, Ladoke Akintola University of Technology, P.M.B. 4000, Ogbomoso, Nigeria.

[2]Department of Chemical Pathology, Ladoke Akintola University of Technology, Teaching Hospital, P.M.B. 4007, Ogbomoso, Nigeria.

[3]Department of Physiology, Ekiti State University, Ado Ekiti, Nigeria.

[4]Histopathology Department, National Hospital, Abuja, Nigeria.

Article Received on
26 January 2018,
Revised on 17 Feb. 2018,
Accepted on 08 March 2018,
DOI: 10.20959/wjpps20184-11252

***Corresponding Author**
Dr. Ajayi Ayodeji Folorunsho
Department of Physiology, Ladoke Akintola University of Technology, P.M.B. 4000, Ogbomoso, Nigeria.

ABSTRACT

Thyroid hormones are vital to regulating growth, calorigenesis and metabolic rate throughout life. They act on almost all organs throughout the body, including the gut and visceral, therefore disturbances in thyroid function may have gastrointestinal manifestations and their effects on body tissues have concomitant influence on the immune system. Interleukin-6 (IL-6) is a pro-inflammatory cytokine which increases with gastric mucosa damage, while, cluster differential (CD3) and cluster differential (CD20) are markers of cell-mediated and humoral-mediated immune responses respectively. This study, therefore, examined the expression of CD3 and CD20 in gastric mucosa with the changes in serum concentration of IL-6 in response to altered thyroid state. The thyroid status of 30 male Wistar rats was altered using carbimazole (hypothyroid) and levothyroxine (hyperthyroid) and the serum interleukin-6 (IL-6), as well as gastric mucosa CD3 and CD20 expression, were assessed. It was observed that altered thyroid state was established with the treatments given. The serum IL-6 increased significantly ($p<0.01$) from 4.0 ng/ml in control to 27.0 ng/ml in hyperthyroid and 56.3 ng/ml in hypothyroid groups. This was associated with an increased CD3 expression in both hypothyroid and hyperthyroid groups compared to the control, while increased CD20 expression was restricted to hypothyroid rats only. Findings from this study demonstrate that

alteration of thyroid state is associated with inflammatory response with consequent immunomodulatory effect on both cells mediated- and humoral mediated immune responses.

KEYWORDS: Cluster Differential; Hyperthyroid; Hypothyroid; Interleukin-6; Thyroid hormones.

INTRODUCTION

Thyroid hormones are important in regulating growth, calorigenesis and metabolic rate throughout life. They act on almost all organs throughout the body, including the gut and visceral, therefore disturbances in thyroid function may have gastrointestinal manifestations.[1] They also have important influences on immunological response of mammals, with various studies indicating their immunomodulatory effects in mammals.[2,3,4] Chatterjee and Chandel reported that administration of thyroid hormones to normal animals induce leukocytosis.[2] Thyroid hormones affect the functional or developmental activity of bone marrow cells and also cells in secondary lymphoid tissues. Reports show that in severe hypothyroidism due to thyrotropin receptor (TSHR) defect, there is impairment of B cell development.[5,6] Previous studies have reported oxidative stress in hyperthyroidism and hypothyroidism.[7,8,9,10,11] This leads to an inflammatory response resulting in increased release of Interleukin-6 (IL-6), a cytokine shown to have a modulating effect on certain endocrine functions.[12]

The CD3 T-cell co-receptor helps to activate the cytotoxic T-Cell. It consists of a protein complex of four distinct chains - a$\underline{\gamma}$ chain, a $\underline{\delta}$ chain, and two $\underline{\varepsilon}$ chains; and forms a portion of the TCR complex and is therefore important in cell-mediated immune response. CD20, on the other hand, is an activated-glycosylated phosphoprotein expressed on the surface of all B-cells beginning at the pro-B phase (CD45R+, CD117+) which progressively increases in concentration until maturity,[13] consequently, CD20 is important in humoral-mediated immune response.

Reports on CD3 and CD20 expression in dysthyroid gastric mucosa are scarce; this study was therefore designed to assess the expression of CD3 and CD20 in gastric mucosa and also to determine changes in serum concentration of IL-6 in dysthyroid rats.

MATERIALS AND METHODS

Experimental animals

Thirty (30) male Wistar rats were obtained from the Animal Holding Unit of the Department of Physiology, Ladoke Akintola University of Technology, Ogbomoso. The rats were apparently healthy, weighing between 160 – 200 grams and had not been subjected to previous experimental procedures. During the study, the animals were kept in wire mesh cages with *ad libitum* access to rat pellet and water. The laboratory temperature was about 24°C and the animals were exposed to normal day/night cycles for seven (7) days before commencement of the experiment.

Treatments

Rats were randomly allocated into three groups: control, hypothyroid, and hyperthyroid and the experiment was carried out, as shown in Table 1 below, as previously described.[14] The experimental study was in accordance with the Institution's guidelines and criteria for humane care outlined in the National Health Guidelines for the care and use of Laboratory Animals. Ethical approval for the implementation of the research clearance was obtained from the Oyo State Research Ethical Review Committee, Ministry of Health Secretariat, Ibadan, Nigeria. The reference number of the ethical approval for this research is AD13/479/143.

Table 1: Experimental protocol

	Control	Hypothyroid	Hyperthyroid
Treatment	Distilled water	5mg/250g body weight of Carbimazole	5µg/100g body weight of levothyroxine
Animals	10 male rats	10 male rats	10 male rats
Duration	30 days	30 days	30 days

Specimen collection

At the end of the experimental period, the rats were sacrificed via cervical dislocation and blood was collected via cardiac puncture into plain bottles, serum was extracted and used to assay for thyroid stimulating hormone (TSH), and IL-6. The stomachs were removed, washed three times in ice cold saline and blotted on ash-free filter paper and used for CD3 and CD20 immunohistochemistry study.

Preparation of tissue homogenates

One part of the stomach was weighed and homogenized in phosphate buffered saline (PBS) 50mM (pH 7.4) for estimation of protein content and IL-6 concentration. The crude tissue

homogenate was centrifuged at 10,000 rpm for 15 minutes at 4°C and the resulting supernatant was used for the various investigations. Protein content in tissue homogenate was measured using Biuret's method while IL-6 concentration was determined using ELISA test Kits manufactured by Elabscience Biotechnology Co. Ltd, Wuhan, China.

ELISA Procedure

Serum concentrations of IL-6 were measured using ELISA kits manufactured by Elabscience Biotechnology Co. Ltd, Wuhan, China. The assays were carried out following manufacturer's instructions. Standard samples were prepared from the calibrator by serial dilution of the calibrator with the diluent concentrate as instructed by the manufacturer. One hundred microliters of the standards and sera/tissue homogenate samples were pipetted into micro wells already coated with specific antibodies and incubated at 37°C for 90 min; the plate was covered during incubation. Following incubation, the wells were aspirated of their contents without washing and 100µl of biotinylated detection antibody was added to each well and incubated for 60 minutes at 37°C, after which each well was completely filled with appropriate wash solution. The plate was washed three times.

One hundred microliters of appropriately diluted enzyme–antibody conjugate was pipetted into each well and the plate was incubated at 37°C for 30 minutes. After incubation, another process of washing was performed as described above and 90µl of TMB substrate solution was added to each well. This was followed by incubation for 15 minutes at 37°C after which 50µl of stop solution was added to each well. The absorbance (at 450 nm) was determined using ELISA reader.

Histological analysis

Histological studies were carried out as previously documented.[14,15] A portion of the stomach was fixed in 10% formal saline for histological examination. The tissues were processed and embedded in paraffin wax. Thick sections (5µm) were obtained and stained by haematoxylin and eosin (H & E) method and examined under a light microscope to determine morphological changes.

Determination of CD3 expression

Expression of CD3 in gastric mucosa cells was determined by immunohistochemistry using anti-rat CD3 antibody in the presence of Streptavidin peroxidase as previously described.[16]

Determination of CD20 Expression

Expression of CD20 in gastric mucosa cells was determined with an immunohistochemistry using anti- rat CD20 antibody in the presence of Streptavidin peroxidase as previously described.[16]

Labeling Index Calculation from ImmunoRatio Web Application.

The percentages of positively stained nuclei for CD3 and CD20 were quantified using Immuno Ratio web application (*http://jvsmicroscope.uta.fi/immunoratio/*) for Image J (http://imagej.nih.gov/ij/). Which resides on a remote server accessed through the internet with a web browser, Its main features include separating diaminobenzidine-stained (DAB) from hematoxylin-stained regions of the image, calculating the percentage of DAB-stained region over total region, which is known as the labeling index and generating a pseudocolored image corresponding with the area segmentation.[17]

Statistical analysis

Data were expressed as the mean ± standard deviation. Data were analyzed using one-way analysis of variance complemented with unpaired *t*-test. Tukey's Multiple Comparison Test was used as *post hoc* test.

RESULTS

Effect of Carbimazole and levothyroxine on Serum TSH

Carbimazole treatment led to significant ($p < 0.01$) increase in serum TSH when compared with the control, while levothyroxine caused a significant ($p < 0.01$) decrease in serum TSH when compared with the control as shown by Table 2.

Table 2: Effect of Carbimazole and levothyroxine treatments on Serum TSH.

Treatments	TSH concentration (ng/dL)
Control	49.0 ± 7.6
Hypothyroid	60.7 ± 3.4*
Hyperthyroid	25.4 ± 2.1#

*Significant difference between hypothyroid and controls ($p< 0.01$)

Significant difference between hyperthyroid and controls ($p< 0.01$)

Effect of carbimazole and levothyroxine on serum IL-6

Carbimazole and levothyroxine treatment in the different groups significantly ($p<0.01$) increased serum IL-6 compared with controls with a greater increase in the carbimazole treated groups compared with levothyroxine treated group (Figure 1).

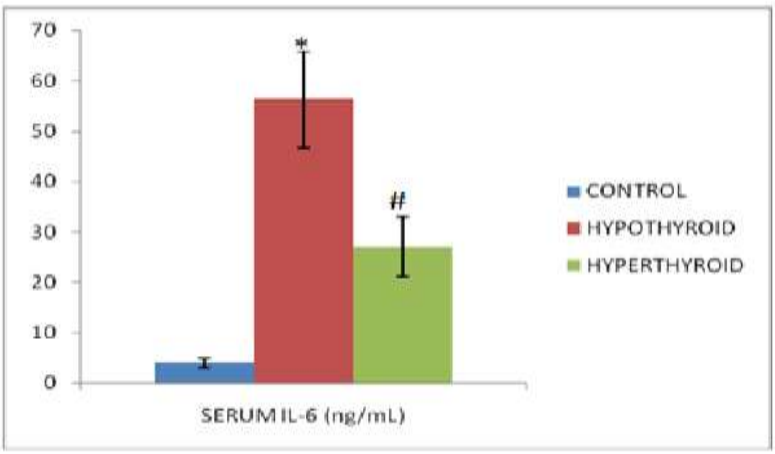

Figure 1: Interleukin (IL)-6 concentrations in serum of rats with altered thyroid states compared with control.

* Significant difference between hypothyroid and controls ($p< 0.01$)

Significant difference between hyperthyroid and controls ($p< 0.01$)

Effect of carbimazole and thyroxine on gastric ulceration

Carbimazole treatment caused ulceration on the gastric mucosa of rats, Thyroxine treatment resulted into inflammation and ulceration of the gastric mucosa, while the untreated rats showed a normal gastric mucosa (Figure 2).

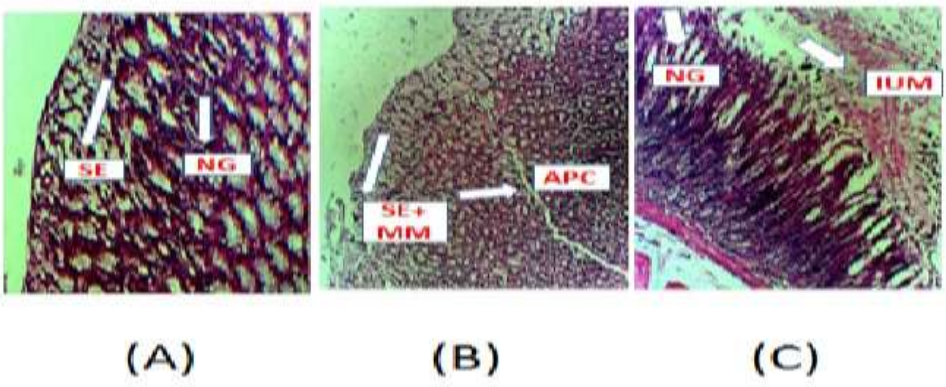

Figure 2: Photomicrograph sections of gastric mucosal surface of control (Section A), hyperthyroid (Section B) and hypothyroid (Section C) rats (H&E X 40).

Legend

Section A: showed normal gastric histology, with intact surface epithelium (SE) and intact neck of glands (NG).

Section B: showed erupted zones on the surface epithelium with presentation of ulceration on the *muscularis mucosa* (SE+MM). Atrophy Parietal cells is also seen (APC).

Section C: showed areas of inflammation and ulceration on the mucosa, but an intact neck of gland (NG).

Effect of carbimazole and levothyroxine on CD3 expression

Carbimazole and levothyroxine treatment in the different groups caused increased CD3 expression in gastric mucosa of treated rats compared with controls (Figure 3).

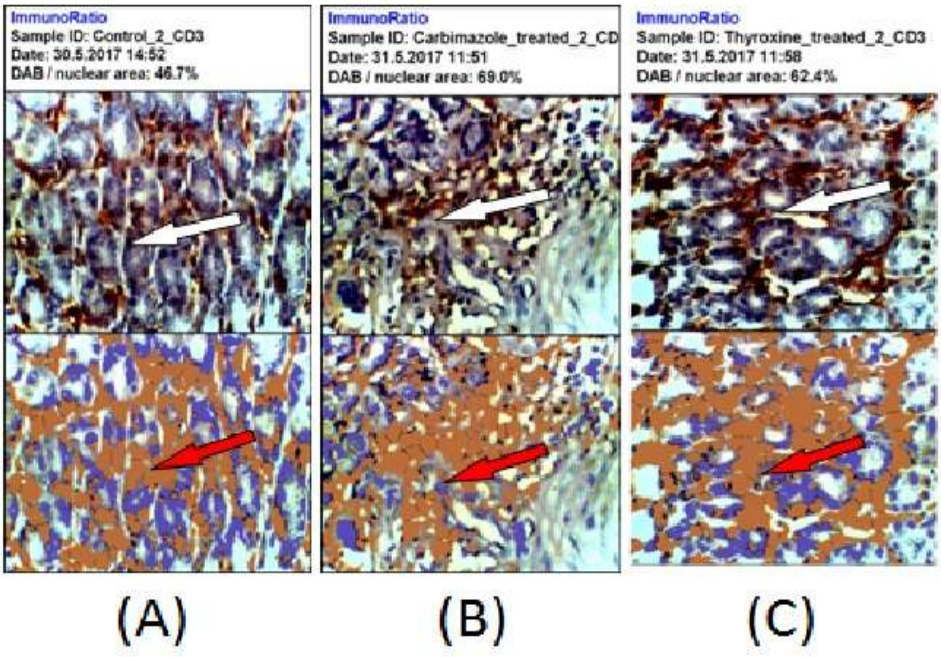

Figure 3: Photmicrograph of CD3 expression on gastric mucosal surface of control, hyperthyroid and hypothyroid rats using immunohistochemistry (X 40 MAG).

(A) Control: White arrow indicates area of mild expression of CD3 on normal gastric mucosa of the control rat's original DAB-stained image. While the red arrow points at pseudo-colored image of control rat's gastric mucosa produced by the ImmunoRatio web application, showing staining components. The labeling index is equal to 46.7%.

(B) Hypothyroid: White arrow indicates area of moderate expression of CD3 on gastric mucosal ulceration of the hypothyroid rat's original DAB-stained image. While the red

arrow points at pseudo-colored image of hypothyroid rat's gastric mucosa produced by the ImmunoRatio web application, showing staining components. The labeling index is equal to 69.0%.

(C) Hyperthyroid: White arrow indicates area of moderate expression of CD3 on gastric mucosal ulceration and sub-mucosa inflammation of the hyperthyroid rat's original DAB-stained image. While the red arrow points at pseudo-colored image of hyperthyroid rat's gastric mucosa produced by the ImmunoRatio web application, showing staining components. The labeling index is equal to 62.4%.

Effect of carbimazole and levothyroxine on CD20 expressions

Carbimazole treatment increased CD-20 expression in gastric mucosa compared with levothyroxine treatment and control expressions (Figure 4).

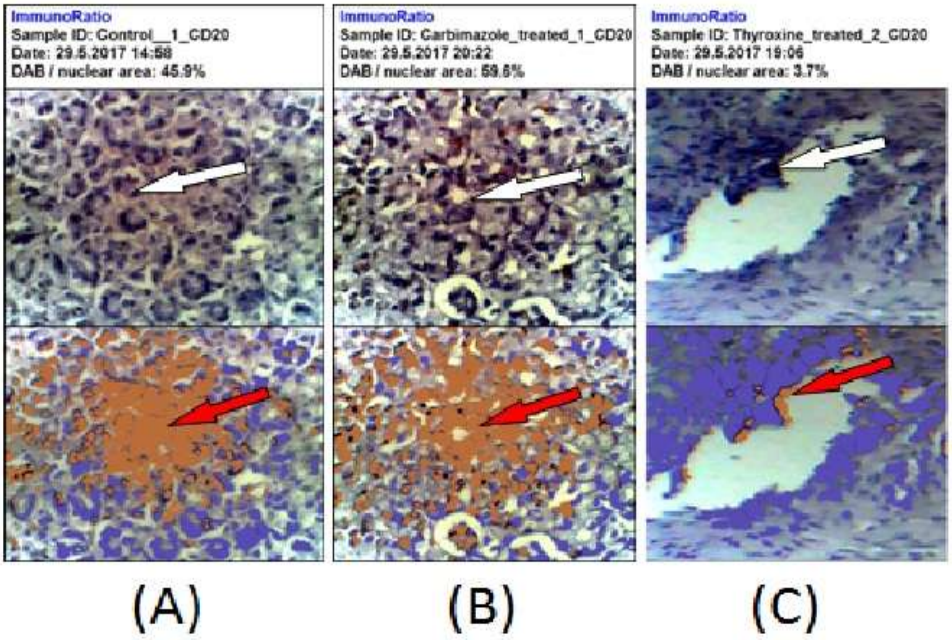

Figure 4: Photmicrograph of CD20 expression on gastric mucosal surface of control, hyperthyroid and hypothyroid rats using immunohistochemistry (X 40 MAG).

(A) Control: White arrow indicates area of mild expression of CD20 on normal gastric mucosa of the control rat's original DAB-stained image. While the red arrow points at pseudo-colored image of control rat's gastric mucosa produced by the ImmunoRatio web application, showing staining components. The labeling index is equal to 45.9%.

(B) Hypothyroid: White arrow indicates area of moderate expression of CD20 on gastric mucosal ulceration of the hypothyroid rat's original DAB-stained image. While the red arrow points at pseudo-colored image of hypothyroid rat's gastric mucosa produced by

the Immuno Ratio web application, showing staining components. The labeling index is equal to 59.6%.

(C) Hyperthyroid: White arrow indicates area of nil expression of CD20 on gastric mucosal ulceration of the hyperthyroid rat's original DAB-stained image. While the red arrow points at pseudo-colored image of hyperthyroid rat's gastric mucosa produced by the Immuno Ratio web application, showing staining components. The labeling index is equal to 3.7%.

DISCUSSION

Thyroid hormones are pleiotropic hormones, with essential roles in growth, differentiation, maturation, and metabolism.[18] Studies abound on the interactions between thyroid hormones and oxidative stress[19,20] and consequent inflammatory response;[21] however, reports are scarce on the effect of 'altered thyroid 'status on expression of CD3 and CD20 in gastric mucosa. We studied the gastric mucosa inflammatory response in rats with experimentally altered thyroid. Treatment with Carbimazole and Levothyroxine treatments in different rats led to hypothyroid and hyperthyroid states, respectively. This was confirmed by measuring serum TSH concentration. Carbimazole significantly increased serum TSH when compared with control, confirming hypothyroid state while levothyroxine significantly decreased serum TSH when compared with control, confirming hyperthyroid state. The increased serum TSH seen in Carbimazole treatment and the decreased serum TSH observed in levothyroxine treatment are due to the negative feedback mechanism along the hypothalamic–pituitary–thyroid axis.[14]

Thyroid hormones affect all organs in the body including the gut and viscera. They also regulate inflammatory responses. Hypermetabolism has been reported to occur as a result of thyroid hormone administration to experimental animals.[22] This hypermetabolic state is accompanied by oxidative stress in several target tissues and consequent inflammatory response.[23, 24] Hypothyroidism is however associated with lower metabolic rate, a decreased free radical production is therefore expected. Interestingly, many studies found that hypothyroidism is associated with oxidative stress[25,26] due to insufficient antioxidant production.[27] Thus, hypothyroidism would also predispose to a pro-inflammatory state.

In this study, a significantly increased serum IL-6 concentration in hyperthyroid and hypothyroid rats was observed. This corroborates the findings of Lakatos *et al*.[28] and Murai *et al*.[29] which observed increased serum IL-6 in hyperthyroidism and hypothyroidism

respectively. This reflects the acute inflammatory response associated with alteration of thyroid status. Also, a moderate expression of CD3 in gastric mucosa cells of hyperthyroid as well as hypothyroid rats was observed. The role of the CD3 receptor has been demonstrated in cell-mediated immunity and especially in the stomach.[30] Focal inflammatory cells were also seen which shows evidence of inflammation. Therefore, our findings suggest that experimental alteration of thyroid status might result in a cell mediated immune response. Furthermore, we also observed a moderate expression of CD20 on gastric mucosa cells of hypothyroid rats which were however not present in hyperthyroid or control rats. CD20 has been shown to have a role in T-dependent humoral mediated immunity,[31] thus our finding might be a humoral immune response to hypothyroidism. Also, the isolated expression of CD20 in gastric mucosa of hypothyroid and not hyperthyroid or control rats also confirms the findings that serum IL-6 is more elevated in hypothyroid compared with hyperthyroid rats.

CONCLUSION

Our findings demonstrate that alteration of thyroid state is associated with an inflammatory response with consequent immunomodulatory effect on both cells mediated- and humoral mediated immune responses. These findings suggest possible studies to explore the specific role of CD3 and CD20 expression in dysthyroid stomach.

REFERENCES

1. Maser C, Toset A, Roman S. Gastrointestinal manifestations of endocrine disease. World J Gastroenterol, 2006; 12: 3174–3179.
2. Chatterjee S, Chandel S. Immunomodulatory role of thyroid hormones: in vivo effect of thyroid hormones on the blastogenic response of lymphoid tissues. Acta Endocrinologica (Copenh), 1983; 103(1): 95-100.
3. Gupta MK, Theresa C, Deodhar SD. Effect of thyroxine on immune response in C57B1/6J mice. Acta Endocrinologica (Copenh), 1983; 103(1): 76-80.
4. Botella-Carretero JI, Prados A, Manzano L, Montero MT, Escribano L, Sancho J, Eschobar-Morreale HF. The effects of thyroid hormones on circulating markers of cell-mediated immune response, as studied in patients with differentiated thyroid carcinoma before and during thyroxine withdrawal. Euro J Endo, 2005; 153(2): 223-230.
5. Foster MP, Montecino-Rodriguez E, Dorshkind K. Proliferation of bone marrow pro-B cells is dependent on stimulation by the pituitary/thyroid axis. J Immunol, 1999; 163: 5883–5889.

6. Dorshkind K, Horseman ND. The roles of prolactin, growth hormone, insulin-like growth factor-I, and thyroid hormones in lymphocyte development and function: Insights from genetic models of hormone and hormone receptor deficiency. Endocrine Rev., 2005; 21: 292–312.
7. Fernandez V, Videla LA. Influence of hyperthyroidism on superoxide radical and hydrogen peroxide production by rat liver submitochondrial. Free Rad Res Commun, 1993; 18: 329-335.
8. Yilmaz S, Ozan S, Benzer F, Canatan H. Oxidative damage and antioxidant enzyme activities in experimental hypothyroidism. Cell Biochem Funct, 2003; 21(4): 325–330.
9. Resch U, Helsel G, Tatzber F, Sinzinger H. Antioxidant status in thyroid dysfunction. Clin Chem Lab Med., 2002; 40: 1132-1134.
10. Chattopadhyay S, Zaidi G, Das K, Chainy GNB. Effects of hypothyroidism induced by 6-n-propylthiouracil and its reversal by T3 on rat heart superoxide dismutase, catalase and lipid peroxidation. Ind J Exp Biol., 2003; 41: 846-849.
11. Pasupathi P, Latha R. Free radical activity and antioxidant defense mechanisms in patients with hypothyroidism. Thyroid Science, 2008; 3(12): CLS1-6.
12. Jilka RL, Hangoc G, Girasole G, Passeri G, Williams DC, Abrams JS, Boyce B, Broxmeyer H, Manolagas SC. Increased osteoclast development after estrogen loss: mediation by interleukin-6. Science, Jul 3, 1992; 257(5066): 88–91.
13. Hardy R. Chapter 7: B-Lymphocyte development and biology. *In*: Fundamental Immunology 6th ed. Philadelphia, Lippincott Williams & Wilkins, 2008; 237–269.
14. Ajayi AF, Akhigbe RE. Antifertility activity of *Cryptolepis sanguinolenta* leaf ethanolic extract in male rats. J Hum Reprod Sci., 2012; 5: 43-47.
15. Ajayi AF, Akhigbe RE, Iyiola TO, Adewumi OM, Olaleye SB. Gastric secretagogue action of *Cryptolepis sanguinolenta* in the perfused stomach of anesthetized rats. Int J Med Biomed Res., 2012; 1: 62-67.
16. Solange R, Daniela G, Manuela B, Marcello G, Piergiuseppe C, Massimo R, Guido C, Silvano B, Immunohistochemical profile and *c-kit*mutations in gastrointestinal stromal tumors. Pathology - Research and Practice, 2005; 2(8): 71-81.
17. Ruifrok AC, Johnston DA. Quantification of histochemical staining by color deconvolution. *Anal Quant* Cytol Histol, 2001; 23: 291-299.
18. Oetting A, Yen PM. New insights into thyroid hormone action. Best Pract Res Clin Endocrinol Metab, 2007; 21: 193–208.

19. Nanda N, Bobby Z, Hamide A, Koner BC, Sridhar MG. "Association between oxidative stress and coronary lipid risk factors in hypothyroid women is independent of body mass index," Metabolism, 2007; 56(10): 1350–1355.
20. Erdamar H, Demirci H, Yaman H. "The effect of hypothyroidism, hyperthyroidism, and their treatment on parameters of oxidative stress and antioxidant status," Clinical Chemistry and Laboratory Medicine, 2008; 46(7): 1004–1010.
21. Mancini A, Di Segni C, Raimondo S, Olivieri G, Silvestrini A, Meucci E, Curro D. Thyroid Hormones, Oxidative stress, and Inflammation. Mediators of Inflammation, 2016; 6757154.
22. Hazzaa S, Badr E, Abdou A. The link between oxidative stress response and tumor necrosis factor-alpha (TNF-alpha) in hepatic tissue of rats with induced thyroid function. J Afr Ass Physiol Sci., 2013; 1(1): 47-54.
23. Venditti P, Pamplona R, Ayala V, De Rosa R, Caldarone G, Di Meo S. Differential effects of experimental and cold-induced hyperthyroidism on factors inducing rat liver oxidative damage. The Journal of Experimental Biology, 2006; 209: 817-825.
24. Aslan M, Cosar N, Celik H, Aksoy N, Dulger A, Begenik H, Soyoral Y, Kucukoglu M, Selek S. Evaluation of oxidative status in patients with hyperthyroidism. Endocrine, 2011; 40: 285–289.
25. Konukoglu D, Ercan M, Hatemi H. Plasma viscosity in female patients with hypothyroidism: effect of oxidative stress and cholesterol. Clinical hemorheology and microcirculation, 2002; 27: 107-113.
26. Torun AN, Kulaksizoglu S, Kulaksizoglu M, Pamuk BO, Isbilen E, Tutuncu NB. "Serum total antioxidant status and lipid peroxidation marker malondialdehyde levels in overt and subclinical hypothyroidism," Clinical Endocrinology, 2008; 70(3): 469–474.
27. Babu K, Jayaraaj IA, Jeganathan P. Effect of Abnormal thyroid hormone changes in lipid peroxidation and Antioxidant imbalance in Hypothyroid and Hyperthyroid patients, Int J Biol Med Res., 2011; 2(4): 1122 – 1126.
28. Lakatos P, Foldes J, Horvath J, Kiss L, Tatrai A, Takacs I, Tarjan G, Stern PH. Serum Interleukin-6 and bone metabolism in patients with thyroid function disorders. J *Clin* Endocrinol Metab, 1997; 82(1): 78-81.
29. Murai H, Murakami S, Ishida K, Sugawara M. Elevated serum interleukin-6 and decreased thyroid hormone levels in postoperative patients and effects of IL-6 on thyroid cell function in vitro. Thyroid, 1996; 6(6): 601-606.

30. Booth JS, Toapanta FR, Salerno-Goncalves R, Patil S, Kader HA, Safta AM, Czinn SJ, Greenwald BD, Sztein MB. Characterization and functional properties of gastric tissue-resident memory T cells from children, adults and the elderly. Front Immunol, 2014; 5: 294.

31. Morsy DE, Sanyal R, Zaiss AK, Deo R, Muruve DA, Deans JP. Reduced T-dependent humoral immunity in CD20-deficient mice. J Immunol, 2013; 191(6): 3112-8.