

The Investigation of Cytokines and Bacterial Isolates in the Patient with Thyroid Gland Disorders

Miald Sajid Hameed^{1*}, Saddam Hussein Jaber²

Department of Biology, College of Education for Pure Science, Ibn Al-Haitham, University of Baghdad, Iraq

*E-mail: hh_love46 [at]yahoo.com

Abstract: The study included the collection of 175 samples from patients diagnosed with thyroid disorder with different ages from during the period from October, 2016 to February, 2017 in Imamin Al-Kadiman medical city and Al-Kindi Teaching Hospital Results found a bacterial growth in the samples of urine, 80% were positive for bacterial growth while 20% were with negative growth for bacterial. All isolated diagnosed by microscopic and morphological characteristics, while API system was used for biochemical detection. The percentage of bacteria in isolates was 50% of *Staphylococcus aureus*, 20% of *Staphylococcus*, 12.5% of both *E. coli* and *Pseudomonas aeruginosa*, 5% of *Serratia marcescens*. The serum levels of IL-6 significantly increased in the patients (341.65 ± 34.44 pg/ml) compared with healthy group (169.62 ± 72.55 pg/ml). Although, TNF- α levels significantly decreased in the patients (228.55 ± 18 pg/ml) compared with healthy group (317.09 ± 24.53 pg/ml). Moreover, a positive correlation was observed between the levels IL-6 and TNF- α with correlation coefficient of 0.452.

Keywords: hyperthyroidism, hypothyroidism, TNF- α , IL-6

1. Introduction

The thyroid gland is an endocrine gland [1] it regulates many metabolic processes and protein synthesis. The thyroid gland secretes thyroid hormones which are controlled by thyroid stimulating hormone (TSH) secreted by pituitary gland; TSH regulate by thyrotropin-releasing hormone (TRH). The thyroid gland secretes three types of hormones: triiodothyronine (T3) and thyroxine (T4) and calcitonin [2] which is important for growth and development. The imbalance of T3 and T4 hormones result in two types of disorders, hyperthyroidism and hypothyroidism compared with the level of TSH [3] additionally; there are two types of hypothyroidism disorder, primary hypothyroidism and secondary hypothyroidism by depending on TSH levels in comparison to T4 and T3 levels. Hyperthyroidism is an increase in the secretion of thyroid hormones T4 and T3 accompanied by a decreasing in TSH [4] The genetic factors are associated with exogenous factors such as smoking, bacterial infections, thus many bacteria have multiple virulence factors such as lipid A, protein A that enables it to penetrate the body defenses and prevents the immune response that activated by immune cells in the host [5] Interleukin-6 (IL-6) and TNF- α are associated with thyroid disorders that play a critical role in autoimmune and inflammatory processes [6].

2. Materials and Methods

This study was conducted in Imamin Al-kadiman medical city and AL-Kindy Teaching hospital. 175 samples were collected from patients diagnosed with thyroid gland disorder and healthy individuals, including 75 blood samples with thyroid disorder and 25 blood samples from healthy individuals as control group. 5 ml of peripheral blood sample was collected from each patient and blood was placed in the test tubes and the serum separated by using a centrifuge at 3000rpm/min for 5 minutes, and then the serum was kept in plastic test tubes at -20° C until using in the immunological and serological assay.

A100 of the median urine samples were collected and stored in sterilized plastic bottles and cultured by striking method on the blood agar base, MacConkey agar and Mannitol salt agar and incubated 37 ° C for 24 hours.

2.1 Diagnosis of bacterial isolates

The bacterial isolates were characterized depending on the morphological features while the biochemical tests assessed by using API20 and API-staph systems.

2.2 Immunological tests

The levels of IL-6 and TNF- α were measured in the serum of patients by Enzyme Linked Immunosorbent (ELISA) method using the kits provided by Prprotech according to the manufacture's procedure and the absorbance was recorded using ELISA spectrophotometer at 405 nm.

3. Results and Discussion

The study included 75 patients diagnosed with thyroid disorder (50 hypothyroidism, 25 hyperthyroidism) and 25 healthy individuals as a control group. The results of the current study revealed there is no significant differences in T3 hormone levels between patients and control group, T3 hormone level was 1.49 ± 0.06 in hypothyroidism patients and it was 1.61 ± 0.10 in control group, whereas the level of hormone T4 decreased in the serum of patients in comparison to its level in serum of control group, as the average level in patients was 73.57 ± 2.85 and 89.68 ± 3.6 in control group. On the other hand, TSH level significantly increased in the serum of patients compared with its level in control group. The mean concentration of TSH in patients was 21.40 ± 3.17 while it was 1.61 ± 0.17 in control group. The results of this study is in agreement with observation of Pluta and others [7] that found increasing in TSH hormone with the reduction of T4. Additionally, the results of this study found that the level of T3 in the serum of hyperthyroidism patients has significantly increased in comparison to its level in control group; the average level

of T3 was 2.45 ± 0.22 and 1.61 ± 0.10 in patients and control respectively. Moreover, T4 significantly increased in the serum of patients (130.04 ± 7.49) compared (89.68 ± 3.69), while the level of TSH significantly decreased in the serum of patients, it was 0.05 ± 0.01 while it was 1.61 ± 0.17 in control group. These results of the current study are agreed with findings of Shupnik [8] with increasing of T3 and T4 and reduction of TSH (table 1, 2; figure 1, 2).

Table1: The levels of T3, T4, TSH levels in the serum of hypothyroidism patients and healthy individuals

Groups	T3(nmol)	T4(nmol)	TSH(nmol)
	Mean±SE	Mean±SE	Mean±SE
Patients	1.49 ± 0.06	73.57 ± 2.85	21.40 ± 3.17
Healthy	1.61 ± 0.10	89.68 ± 3.6	1.61 ± 0.17

Table2: The levels of T3, T4, TSH levels in the serum of hyperthyroidism patients and healthy individuals

Groups	T3(nmol)	T4(nmol)	TSH(nmol)
	Mean±SE	Mean±SE	Mean±SE
Patients	2.45 ± 0.22	130.04 ± 7.49	0.05 ± 0.01
Healthy	1.61 ± 0.10	89.68 ± 3.69	1.61 ± 0.17

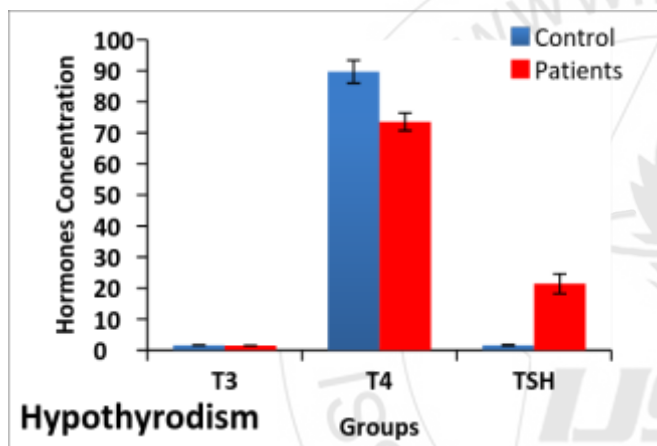


Figure 1: The levels of T3, T4, TSH levels in the serum of hypothyroidism patients

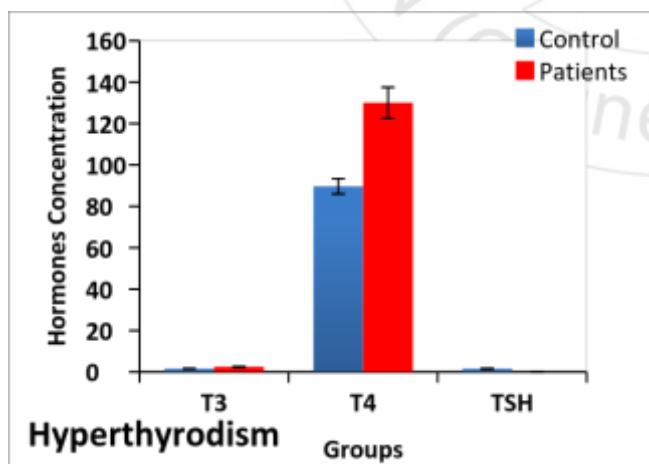


Figure2: The levels of T3, T4, TSH levels in the serum of hyperthyroidism patients

The percentage of bacterial tests was 80% of positive samples and 20% of negative samples, and the positive samples were with 50% of *S. aureus*, 20% of *S. saprophyticus*, 12.5% of *E. coli* and *P. aeruginosa* and 5% of *Serratia marcescens* (Table 3).

Table 3: The percentage of bacterial isolates

Types of bacteria	Percentage (%)
<i>S. aureus</i>	50%
<i>S. saprophyticus</i>	20%
<i>E. coli</i>	12.5%
<i>P. aeruginosa</i>	12.5%
<i>Serratia.marcescens</i>	5%

These results were is not agree with the findings of Tomer [9] that explained the thyroid gland disorder was caused by genetic and environmental factors, while the results is in agreement with Cappa et al [10] that observed bacterial infections have a critical role in the thyroid disorder.

Moreover, the results revealed that *S. aureus* is the most frequent of bacteria species in samples with 50% due to the presence of many virulence factors including intestinal toxins, somatic antigens, flagella and production of lactation enzyme. The results were differed with the results of the study of Barisic et al. [11] that found the percentage of *S.aureus* was 3.8% in the samples of patients with urinary tract infection patients did not have autoimmune disease.

3.1 Assessment of cytokines in the serum of patients with thyroid disorders

IL-6 and TNF- α concentration were determined in the serum of patients diagnosed with thyroid disorder by using ELISA technique, and the concentration of each one compared with healthy individuals group. IL-6 is proinflammatory cytokines able to stimulate B-cells to produce antibodies and also elevates the concentration of C-reactive protein as one of the acute phase proteins. The results of current study found that IL-6 levels significantly increased in the patient in comparison to healthy group ($P < 0.05$); it was (341.65 ± 34.44 pg/ml) and (169.62 ± 72.55) respectively (Table 4) (Fig.3).

Table 4: Levels of IL-6 in the serum of healthy and patients groups

Cytokine	Group	The number	Mean±S.E.
IL-6	Control	25	169.62 ± 72.55
	Patients	75	341.65 ± 34.44

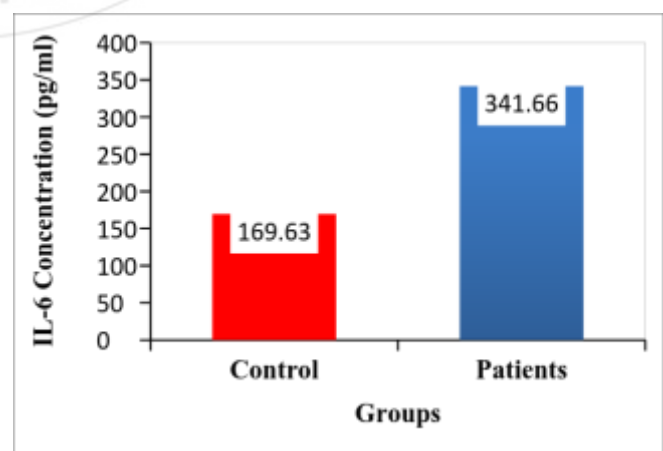


Figure 3: Serum level of IL-6 in the patients and healthy groups

This result was constant with the findings of Bartalena et al. [12] that referred to the significant difference in the serum levels of IL-6 in the patients compared to the healthy individuals.

As shown in Table (5) and Fig. (4), TNF- α significantly decreased in the patients with thyroid disorder which was (317.09 \pm 24.53) compared to control group which was (228.55 \pm 18.37) ($p < 0.05$). This result was in opposite with result of Nielsen et al. [13] that observed a significant increasing in the concentration of TNF- α . The decreasing of TNF- α could explain to the presence of the bacteria that indirectly inhibits the TNF- α -mediated immune response by a reduction in the levels of inflammatory cytokines and modulation of pathways of transcriptional factors such as NF- κ B that implicated in the production of inflammatory cytokines [13]. Interestingly, the statistical analysis of this study observed a significant correlation between IL-6 and TNF- α and the correlation coefficient was 0.452 with significant $p < 0.05$.

Table 5: The levels of TNF- α in the serum of healthy and patients groups

Cytokine	Group	The number	Mean \pm S.E.
TNF- α	Control	25	317.09 \pm 24.53
	Patients	75	228.55 \pm 18.37

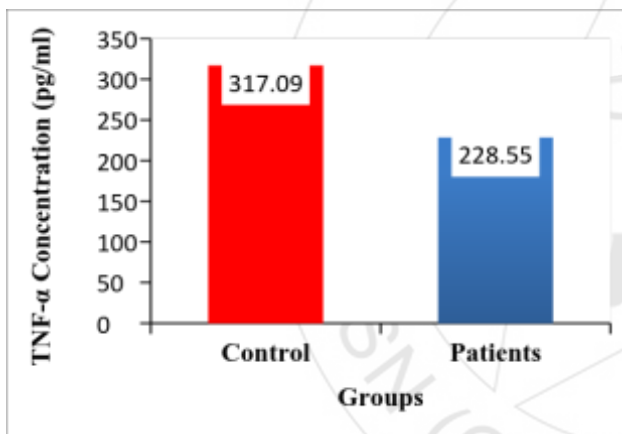


Figure 4: Serum level of TNF- α in the patients and healthy groups

References

[1] Guyton, A.C. and Hall, J.E. Medical physiology text book, 11th ed. Elsevier Saunders, Philadelphia. 881-883, 2011.
 [2] Lynn, W.R and Lynn, J.A. "Hypothyroidism is easily over looked", Practitioner, 22(4):224-231, 2007.
 [3] Saladin, K.S. and Miller, L. Anatomy and physiology the unity of form and function. 3th ed. McGraw-Hill Co. London, 1192pp, 2003.
 [4] Khan, A.M.; Khan, M.A. and Akhtar, S.H. "Thyroid disorder, Etiology and Prevalence", J. Med. Sci., 2(2):89-94, 2002.
 [5] Kenneth, M. Jane ways Immunobiological; 7th ed., 2008.
 [6] Goldsby, R.A.; Kindt, T.J.; Osborne, B.A and Kuby, J. Immunology, 5th ed. W. H. fr eeman and company: USA, 2003.

[7] Pluta, R.M; Writer; A. E. Burke, MA, Illustrator and Richard M. G. "Subclinical Hypothyroidism", JAMA; 304(12): 1402, 2010.
 [8] Shupnik, M.A. "The thyroid gland", Physiology. 704: 82-8, 2003.
 [9] Tomer, Y. "Interferon induced thyroiditis", J Autoimmun, 34(3): 322-326, 2010.
 [10] Cappa, M.; Bizzarri, C. and Crea, F. "Autoimmune Thyroid Disease in Children", J. Thyroid Res.10:13, 2011.
 [11] Barisic, Z.; Babic-Erceg, A., Borzic, E.; Zoranic, V.; Kaliterna, V. and Carev, M. "Urinary tract infections in South Croatia: aetiology and antimicrobial resistance", Int. J. Antimicrob. Agen., 2:61-64, 2003.
 [12] Bartalena, L.; Grasso, L.; Brogion, S.; Aghini-lombardi, F.; Braveman, L.E. and Martin, E. "Serum interleukin-6 in aminodarone-induced thyrotoxicosis", J. Clin. Endocrinol. Metab., 78: 423-427, 1994.
 [13] Selveeraj, S.K. and Prasadarao, N.V. "Escherichia coli K1 inhibiting of proinflammatory cytokine induction in monocyte by preventing NF-kappa B activation", J Leuko. Biol. 78:544-554, 2005