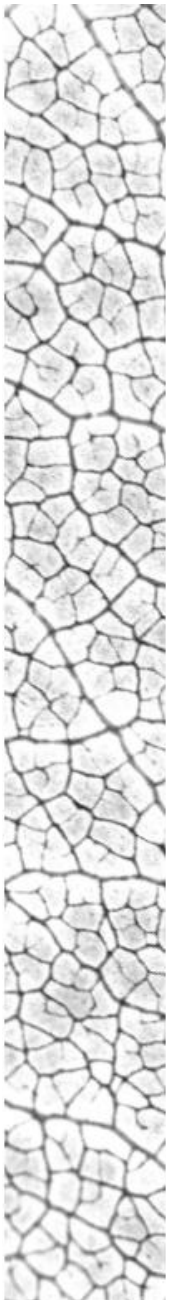

EurAsian Journal of BioSciences
Eurasia J Biosci 14, 4645-4648 (2020)

Investigation of interleukin 12 (IL-12) in graves' disease of Iraqi patients by using ELIZA

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Abstract
Cytokines are likely to play a vital role in autoimmune thyroid disease. These molecules, produced by both infiltrating inflammatory cells and thyroid follicular cells (TFC) Interleukin-12 was found to be an immunoregulatory cytokine, which may provide an important link between nonspecific immune mechanisms and the development of a specific T-cell-mediated immune response. The pathogenesis of Graves' disease is thought to be related to TRAb (Thyroid receptor antibody and Thyroid stimulating hormone [TSH]), which is commonly used as an immunological parameter. It is also conceivable that serum-soluble IL-12R (sIL-12R) is a marker of T-cell activation in various autoimmune diseases, and although many studies have found increased levels of serum sIL-12R in Graves' disease, the role of IL-12 has not been investigated sufficiently.
Object: To investigate a role of IL-12 in Iraqi patients with autoimmune thyroid disease.
Subject and methods: This study comprised 45 patients there were 14 men and 31 women aged from 23 to 75 years old (average age, 47.11 years old).All patients had been clinically thyrotoxic, with diffusely enlarged thyroid glands,14 age and sex matched healthy subject with no case history of graves' disease served as an impact group. The serum stored at -4°C for the quantitative assessment of IL-12 using enzyme-linked immunosorbent assay (ELISA) technique (Elabscience Human IL-12 ELISA kit).
Results: The levels of serum IL-12 were higher in the patients with Graves' disease than those in the controls. The levels of serum IL-12 were significantly correlated with the levels of free T4 and TSH. On the other hand, there was no significant relationship between the levels of serum IL-12 and the level free T3 in the patients with Graves' disease.
Conclusion: Measurement of the levels of serum IL-12 may be consider a useful immunological marker during the time course of treatment for Graves' disease as an autoimmune disorder.

Keywords: IL12, graves' disease, health, toxicity

Ali HS, Fahad HM, Jasim MB (2020) Investigation of interleukin 12 (IL-12) in graves' disease of Iraqi patients by using ELIZA. Eurasia J Biosci 14: 4645-4648.

INTRODUCTION

Cytokines are likely to play a vital role in autoimmune thyroid disease. These molecules, produced by both infiltrating inflammatory cells and thyroid follicular cells (TFC). (Del Prete et al. 1986; Zheng et al. 1992) Are essential for T and B cell growth and differentiation, and may affect thyroid follicular cells directly, inducing expression of major histocompatibility complex (MHC) class II molecules and adhesion molecules (Todd et al., 1985; Vargas et al. 1994).

Interleukin-12 (IL-12) was originally identified as a natural killer (NK) cell stimulatory factor. It is a disulfide-linked heterodimeric cytokine composed of 35 kDa and 40 kDa subunits, which is secreted principally by antigen-presenting cells (APC), such as macrophages, some B cells, and dendritic cells. Interleukin-12 activates NK cells and T cells to produce interferon-g and augments their cytotoxic activity and proliferation.

Interleukin-12 was also found to be an immuno-regulatory cytokine, which may provide an important link between nonspecific immune mechanisms and the development of a specific T-cell-mediated immune response. In particular, it influences the development of a T-helper type 1 (Th1), which produces INF-γ, interleukin-2 (IL-2), and tumor necrosis factor- β (Murakami et al. 2005).

Graves' disease is regarded as an autoimmune disease, and has been the subject of many clinical and experimental studies.(Weetman and McGregor, 1994)The pathogenesis of Graves' disease is thought to be related to TRAb (thyroidstimulating hormone [TSH] receptor antibody), which is commonly used as an

Received: February 2020
Accepted: March 2020
Printed: October 2020

immunological parameter. It is also conceivable that serum-soluble IL-2R (sIL-2R) is a marker of T-cell activation in various autoimmune diseases, and although many studies have found increased levels of serum sIL-2R in Graves' disease, the role of IL-12 has not been investigated sufficiently (Murakami et al. 2005; Vidinov and Stoinov, 2019).

IL-12 has not been studied sufficiently in autoimmune thyroid disease in Iraqi patients. To investigate a role of IL-12 in autoimmune thyroid disease, the serum IL-12 levels were measured A standard curve plotted and the results were calculated.in various functional states of autoimmune thyroid disease

MATERIALS AND METHODS

Subject and study

This study comprised 45 patients who were admitted forspecialist center for endocrinology and diabetic disease. There were 14 men and 31 women aged from 23 to 75 years old (average age, 47.11 years old).Additionally, 14 age and sex matched healthy subject with no case history of graves' disease served as an impact group**.**All patients had been clinically thyrotoxic, with diffusely enlarged thyroid glands. Ethical approval was obtained from the local research ethics; all subjects gave an informed written consent before the study.Venous blood was collected from all of these patients and the serum was.

The serum stored at -4°C for the quantitative assessment of IL-12 using enzyme linked immunosorbent assay (ELISA) technique (Elabscience Human IL-12 EASIA kit).Samples were brought to room temperature and processed according to manufacturer's description. Color detection was read at 450 nm Correcting for optical imperfections was done at 630 nm. A standard curve was plotted and results were calculated.

The procedure

1. Add the Standard working solution to the first two columns: Each concentration of the solution is added in duplicate, to one well each, side-by-side (100 uL for each well). Add the samples to other wells (100 uL for every well). Cover the plate with the sealer provided within the kit. Incubate for 90 min at 37℃. Note: solutions should be added to the underside of the micro ELISA plate well, and Immediately add 100 µL of Biotinylated Detection Ab working solution to every well, And avoid touching the inside wall and causing foaming as much as possible.

2. Remove the liquid out of each well, do not wash. Immediately add 100 µL of Biotinylated Detection Ab working solution to every well. Cover with the Plate sealer. Gently mix up. Incubate for 1 hour at 37°C.

3. Aspirate or decant the solution from each well, add 350 uL of wash buffer to each well. Soak for 1~2 min and aspirate or decant the solution from every well and put it dry against clean absorbent paper. Repeat this wash step 3 times. Note: a microplate washer will be utilized in this step and other wash steps..

4. Add 100 µL of HRP Conjugate working solution to each well. Cover with the Plate sealer. Incubate for 30 min at 37°C.

5. Aspirate or decant the solution from each well, repeat the wash process for five times as conducted in step 3

6. Add 90 µL of Substrate Reagent to each well. Cover with a new plate sealer. Incubate for about 15 min at 37°C. Protect the plate from light. Note: the reaction is shortened or extended per the particular color change, but less than 30min.

7. Add 50 µL of Stop Solution to each well. Note: Adding the stop solution should be done as the order like the substrate solution.

8. Determine the optical density (OD value) of each well at once with a micro-plate reader set to 450 nm.

Calculation

The sample results were calculated by interpolation from a typical curve that was performed within the same assay as that for the sample employing a curve fitting equation

Table 1. The mean of interleukin level in TSH patients group compared to control group

IL12 level mean ± SE		Probability
Patients group	Control group	
47.45 ± 21.81	18.89 ± 3.69	P < 0.05

Table 2. The mean of Hormones level in thyrodisim patients group compared to control group

Hormones level mean ± SE			Probability
T3	Patients group	Control group	
	1.69 ± 0.16	1.51 ± 0.10	P > 0.05
T4	109.80± 8.53	73.81± 3.03	P < 0.05
TSH	7.29 ± 1.45	2.87 ± 0.34	P < 0.05

RESULTS

The preoperative levels of serum IL-12 were higher in the patients with Graves' disease than those in the controls as explained in **Table 1**. While **Table 2** shows the level of hormones (T4 and TSH) respectively were significant higher in patients than healthy controls. The levels of serum IL-12 were significantly correlated with the levels of free T4 and TSH, or the weight of the goiter. On the other hand, there was no significant relationship between the levels of serum IL-12 and free T3 as showed in **Table 3**. The relation between the IL-12 level and free T4 and TSH, and T3 non-significant positive and negative as mentioned in **Table 3**.

DISCUSSION

A role of IL-12 in autoimmune thyroid disease is uncertain. The most effective inducers of IL-12 production are bacteria, bacterial products (e.g.

Table 3. The relation between Hormones level and IL-12 level

		IL	T3	T4	TSH
IL	Pearson Correlation	1	0.016	-0.236	0.126
	Sig. (2-tailed)		0.934	0.361	0.556
T3	Pearson Correlation	0.016	1	-0.349	-0.282
	Sig. (2-tailed)	0.934		0.170	0.181
T4	Pearson Correlation	-0.236	-0.349	1	0.071
	Sig. (2-tailed)	0.361	0.170		0.846
TSH	Pearson Correlation	0.126	-0.282	0.071	1
	Sig. (2-tailed)	0.556	0.181	0.846	

Staphylococcus aureus Cowan strain I, lipopolysaccharide), certain viruses (e.g. Herpes simplex virus) and intracellular parasites (e.g. Toxoplasma) (Trinchieri, 1995; Halpern, Kurlander and Pisetsky, 1996). Interleukin-12, which is produced primarily by activated monocytes, is an immune-regulatory cytokine. It initiates and assists in the development of a specific T-cell-mediated immune response. In particular, IL-12 enhances the proliferation, cytokine production, and cytotoxic activity of the T lymphocytes (2-2) Moreover, in vitro and in vivo studies have shown that IL-12 induces the development of Th1 cells, which suggests that it might play an important role in the generation of Th1- mediated autoimmune disease (Trembleau et al.1995; Seder et al. 1996).

Based on a model of experimental autoimmune encephalomyelitis, Leonard et al. suggested that IL-12 might play a pivotal role in the pathogenesis of this autoimmune disease (Leonard et al. 1995). As antibodies to IL-12 abrogated established experimental colitis in mice, it is conceivable that IL-12 could activate inflammatory bowel diseases such as Crohn's disease (Neurath et al.1995).

Some reports have suggested that IL-12 is involved in the pathogenesis and development of Graves' disease, as an autoimmune disease.(Jones et al. 2000; Nagayama et al. 2003), and this agree with our study results there's a significant relation between the IL-12 level and untreated Graves' disease. These results suggest that thyroid hormone by itself might cause a rise in IL-12 levels, which this effect could be caused by chronic stimulation with thyroid hormone, and this accept as true with other study by Murakami et al. (2005).

Regarding the relation of IL-12 and thyroid disease, Zipris et al. of IL-12 and thyroid disease, Zipris et al. have shown in Bio Breeding (BB) autoimmune thyroiditisprone rats that IL-12 p40 gene expression increased in the thyroid gland with the progression of thyroiditis, implicating IL-12 in the perpetuation, if not the initiation, of the disease (Zipris et al., 1996).Therefore, we do not think that IL-12 itself initiates Graves' disease although it may influence its progression.

The level of the free thyroid hormones (T3, T4, and TSH) in patients compared to control was ($p>0.05$) for T3 and ($p<0.05$) for T4 and TSH respectively.

CONCLUSION

Measurement of the levels of serum IL-12 may be consider a useful immunological marker during the time course of treatment for Graves' disease as an autoimmune disorder.

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