Original Article

Evaluation of the new marker interleukin - 33 in Iraqi female patients with hyperthyroidism.

Bushra H. Ali* **MSc, PhD. Biochemistery**

Summary:

Background: Hyperthyroidism refers to overactive of thyroid gland leading to excessive synthesis of thyroid hormones and accelerated metabolism in the peripheral tissue.

Objective: The aim of this study is to evaluate a new member of the IL-1 super family of cytokines interleukin-33(IL-33) levels in serum .in order to evaluate its utility as clinical bio marker of autoimmune disease (i.e. hyperthyroidism)

Methods: The present study was conducted on 30 patients from the Iraqi female patients with hyperthyroidism attending Baghdad teaching hospital, in addition to 30 healthy controls. All subjects were (35-65) years old. Parameters measured in the sera of patients and healthy groups, were interleukin -33 (IL-33), Thyroxin (T4), Thyroxin (T3) and Thyroid stimulating hormone TSH.

Results: A new member of super family cytokines Interleukin -33 was determined in hyperthyroidism, female patients. Higher significant elevation was found when compared with healthy control.

Conclusion: From this study a conclusion was drawn, that evaluation of concentration of a new super family cytokines IL-33 could consider as a clinical biomarkers in hyperthyroidism female patients. This finding may indicate that autoimmune disease like hyperthyroidism might influence cytokine like interleukin -33 production in these patients

Key word: Hyperthyroidism, Interlukin-33, Thyroxin, Thyroid stimulating hormone.

JFac Med Baghdad 2013; Vol.55, No .2
Received Jan .2013
Accepted May.2013

Introduction:

Hyperthyroidism, often referred to as an 'overactive thyroid', is when the thyroid gland produces and secretes excessive amounts of the free, unbound circulating thyroid hormones into e-blood (1). Thyroid hormones are, Triiodothyronine (T3) and or thyroxin (T4) (2). Hyperthyroidism is a type thyrotoxicosis, a hype metabolic clinical syndrome which occurs when there are elevated serum levels of T3 and or T4 (3, 4).Grave's disease is the most common form of hyperthyroidism. (5) .Thyroid imaging and iotracer thyroid uptake measurements, combined with serologic data, enable specific diagnosis and appropriate patient treatment (6). A thyroid- stimulating hormone (TSH) blood test is used to check for thyroid gland problems,TSH is produced when the hypothalamus releases a substance called thyrotropin-releasing hormone (TRH), triggers the pituitary gland to release TSH, the later causes the thyroid gland to make the two hormones: the T3 and T4 in order to control body's metabolism .Both hormones are needed for normal growth of the brain, especially during the first 3 years of life. A baby whose thyroid gland does not make enough thyroid hormone (congenital hypothyroidism) may, in severe cases, be mentally retarded. Older children also need thyroid hormones to grow and develop normally (7).Interleukins are a subset of a larger group of cellular messenger molecules called cytokines , which are modulators of cellular behavior. Like other cytokines, interleukins are not stored within cells but are instead secreted rapidly, and briefly, in response to a stimulus, such as an infectious agent. Once an interleukin has been produced, it travels to its target cell and binds to it via a receptor molecule on the cell's surface. This interaction triggers a cascade of signals within the target cell that ultimately alter the cell's behavior. (8) Interleukins regulate immune responses. (9). Interleukin (IL)-33 is a new member of the IL-1 superfamily of cytokines that is expressed mainly by stromal cells, such as epithelial and endothelial cells, and its expression is up regulated following pro-inflammatory stimulation (10) IL-33 one of a group of related proteins made by leukocytes (white blood cells) and other cells in the body. The set of interleukins stimulated by a specific infectious agent determines which cells will respond to the infection and influences some of the clinical manifestations of the disease (11).IL-33 induced T cells to produce type 2 cytokine (12). IL-33 can function both as a traditional cytokine and as a nuclear factor regulating gene transcription. It is thought to function as an 'alarm in' released following cell necrosis to alerting the immune system to tissue damage or stress. It mediates its biological effects via interaction with the receptors Interleukin 1 receptor-like 1 (IL-1RL1) ST2 and IL-1 receptor accessory protein (IL-1RAcP), both of which are widely expressed, particularly by innate immune cells and T helper 2 (Th2) cells. IL-33 strongly induces Th2 cytokine production from these cells and can promote the pathogenesis of Th2-related disease such as asthma, atopic dermatitis and anaphylaxis. However, IL-33 has shown various protective effects in cardiovascular diseases such as atherosclerosis, obesity, type 2 diabetes and cardiac remodeling. Thus, the effects of IL-33 are either pro-

*Department of chemistry Ibn – Alhaitham / College of Pure Sciences Education / University of Baghdad.

or anti-inflammatory depending on the disease and the model (10).

Experimental Part:

Subjects

The present study was performed on a group of 30 human from Baghdad of Iraqi females patients with hyperthyroidism during April –September 2012 .They were diagnosed by physician at the hospital using x-ray examination . In addition, a group of 30 healthy females were enrolled in the study as a control group.

Sampling

Blood samples of 5ml were drawn from all subjects enrolled in this study, and kept in plain tubes left to clot at room temperature for 15 min. Then centrifuged at 3500 g for 10 min to separate the serum Interleukin – 33 (IL-33) determinations:- Interleukin 33(IL-33) has been estimated by using enzyme immunoassay (ELISA) technique using the manufacturer instruction as supplied with kit from Ray Bio® Human Thyroid stimulating hormone (TSH), T4, and T3 determinations. The procedure was done according to the manufactured instruction as kit supplied from CE (ST AIA-PACK TSH, T3, and T4).

Statistical analysis:-

The results were expressed as Mean± SD. Student-test was used to show the difference between groups variation was considered significant when P-values are ≤ 0.05. The correlation coefficient (r) test is used to describe the association between the different studied parameters.

Results:

Table (1) shows the levels of IL-33 concentration are (230.02± 98.38 Pg/ ml), (14.28± 10.64 Pg/ml) in sera of patients and control respectively. This table shows significant increase in females patients compared with the healthy control was for IL-33 and also significant increase in T3, T4 but decreased in TSH concentration. While they were non significant negative (-ve) correlation between IL-33 and T3($r=-0.29$, $p>0.05$) so as the correlation between IL-33 and T4 ($r= -0.40$, $p>0.05$) as shown in figures (1), (2) espectively, while there was a significant positive (+ve) correlation ($r=0.02$, $p<0.05$) in figure (3) for control group. So that we show highly significant positive (+ve) correlation between IL-33and T3($r=0.12$, $p< 0.05$) so with IL-33 and TSH ($r=0.39$, $p< 0.05$) for patients group in figure (4) and figure (6), but we show highly significant negative ($r=-0.30$, $p<0.05$) correlation between IL-33 and T4in figure (5) for patients group.

Table (1):-The Concentration of (mean ± SD) for IL-33, T3, T 4 and TSH level in patient and control group

Groups Parameters	Controls n=30 Mean± SD	Patients n=30 Mean± SD	P value
IL-33 Pg/ml	14.28±10.64	230.02±98.38	0.05≤
T3ng/ml	2.66±0.37	9.45±1.40	0.05≤
T4µg/dl	1.22±0.28	13.21±0.52	0.05≤
TSH µU/ml	0.49±0.01	0.29±0.01	≤0.05

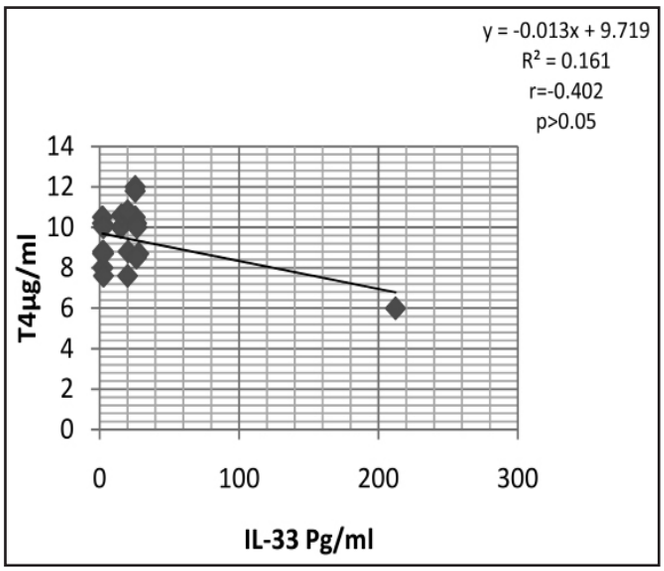

Figure (1):- Correlation between IL-33 and T3 in control group

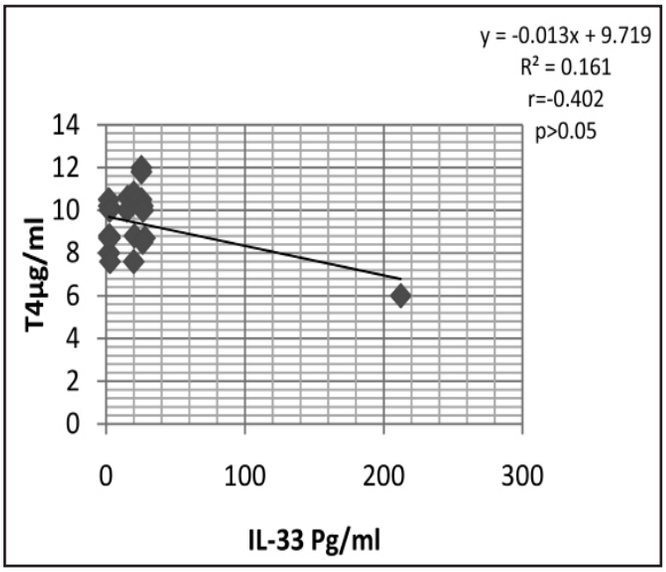

Figure (2):- Correlation between IL-33 and T4 in control group

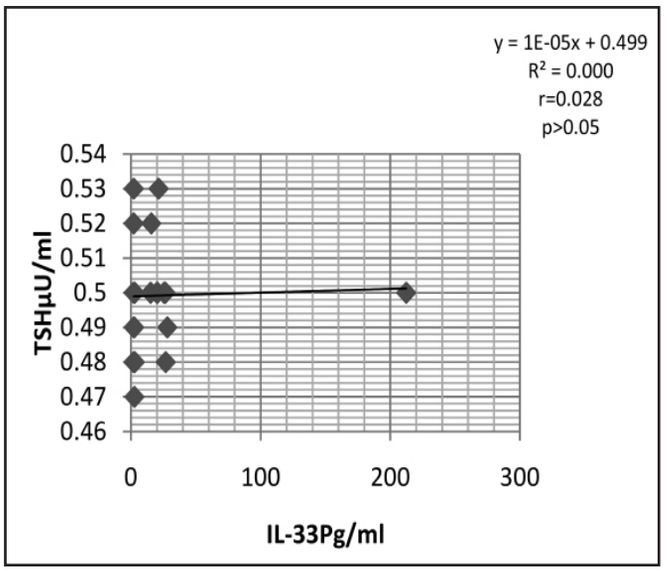

Figure (3):- Correlation between IL-33 and TSH in control group

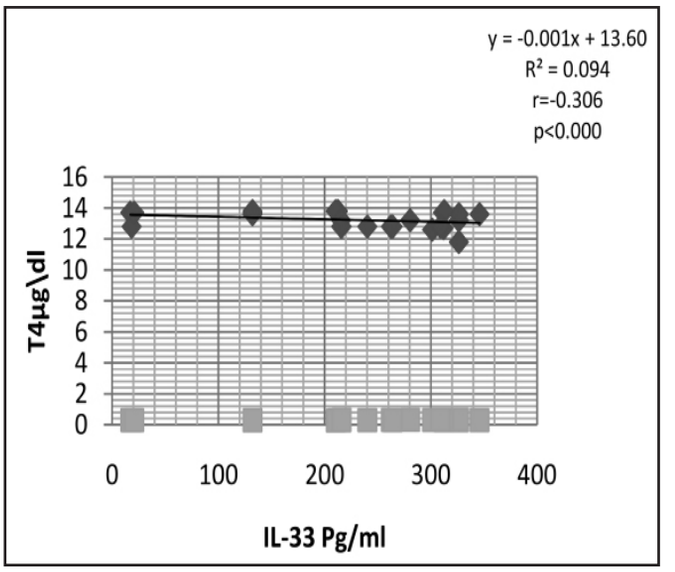

Figure (5):- Correlation between IL-33 and T4 in patients group

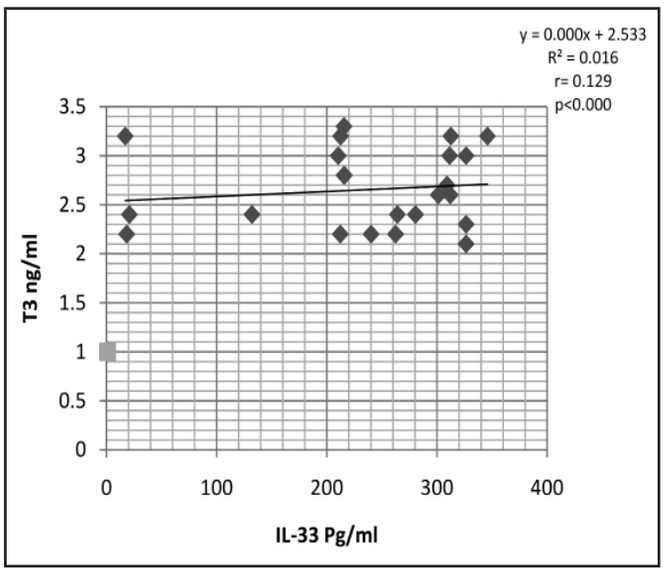

Figure (4):- Correlation between IL-33 and T3 in patients group

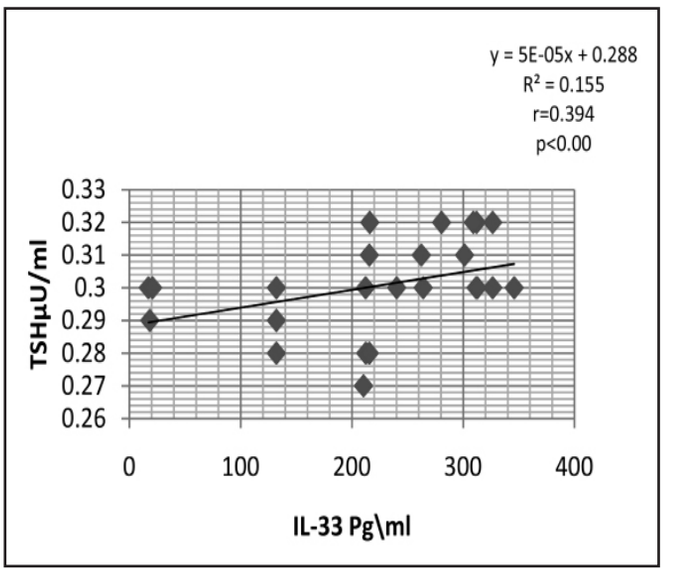

Figure (6):- Correlation between IL-33 and TSH in patients group

Discussion:

The results in the present study showed that the serum level of IL – 33 was significantly higher in female patients with autoimmune disease (hyperthyroidism) than in healthy control .No data in the literature was found concerning the level of IL-33 in such patients. But other study suggest that cytokine-mediated immunity plays a crucial role in the pathogenesis of various autoimmune diseases, including Rheumatoid Arthritis (RA) (13) .Recently, the IL-1-family-related cytokine, IL-33, was detected at high levels in experimental inflammatory arthritis and in the early phase of human RA, and was reported to exert profound pro-inflammatory effects in several experimental autoimmune models(14,15). Moreover, administration of IL-33 leads to the development of severe inflammatory arthritis, suggesting that IL-33 may be therapeutically relevant in RA and the targeting of IL-33 or the IL-33 receptor has been proposed as a potential therapeutic approach for autoimmune diseases such as RA. The biological features of IL-33 and summarize recent advances in understanding of the role of IL-33 in the pathogenesis and treatment of RA. It is hoped that this information may aid the development of novel therapeutic strategies for RA (16). While other study suggests that locally produced IL-33 may contribute to the pathogenesis of joint inflammation and destruction (17). Hyperthyroidism

includes diseases that are a subset of thyrotoxicosis, caused by excess synthesis and secretion of thyroid hormone. Usual causes of hyperthyroidism are Graves' disease in the young and the middle aged, and multinodular goiter in the elderly. Thyrotoxicosis can exist without hyperthyroidism, e.g. exogenous thyroid hormone intake and thyroiditis. Increased serum interleukin-6 (IL-6) concentrations in hyperthyroid patients may also play a role in thyroid hormone-stimulated bone loss. Interleukin-6 stimulates osteoblast production and may be effectors of the action of parathyroid hormone (PTH) on bone (18). Elevated serum sST2 level in Systemic Lupus Erythematosus (SLE) patients was found to correlate with disease activity and was sensitive to change, suggesting a potential role as a surrogate marker of disease activity (19) .Interleukin 33 (IL-33) is a newly described member of the IL-1 super family of cytokines, that could play a crucial role in the pathogenesis of hyperthyroidism through its mediated of T-cell in the immune responses (20) For many years, the interleukin-1 receptor family member ST2 was an orphan receptor that was studied in the context of inflammatory and autoimmune disease. However, in 2005, a new cytokine--interleukin-33 (IL-33)--was identified as a functional ligand for Toll-interleukin -1receptor superfamily (ST2). IL-33/ST2 signaling is involved in T-cell mediated immune responses. Recently, an unanticipated role in cardiovascular disease has been demonstrated, IL-33/ST2 not only represents a promising cardiovascular biomarker but also a novel mechanism of intra myocardial fibroblast-cardiomyocyte communication that may prove to be a therapeutic target for the prevention of heart failure.(21) . On the other hand, several studies have reported on the inhibitory effects of sST2 in inflammatory and fibrotic diseases, suggesting that IL-33/ST2 is a unique cytokine with potential pro- and anti-inflammatory effects (22) Many previous studies have demonstrated that IL-33 may have a pleiotropic function in different diseases, and it could represent a novel target for the treatment of a range of diseases. Recent works have explored the role of IL-33 in chronic autoimmune diseases, such as systemic lerosis, in lammatory bowel disease, rheumatoid arthritis, and systemic lupus erythematosus. These results indicate that IL-33 may contribute to the pathogenesis of chronic autoimmune diseases. (23) IL-33 has a protective role in atherosclerosis by reducing macrophage foam cell formation suggesting that IL-33 may to be a potential therapeutic agent against atherosclerosis. [21] Furthermore, experimental data suggest a protective role for ST2 during Acute Pancreatitis (AP). Highlighting the potential regulatory role of mast cells and the possibility of the ST2 pathway as a new therapeutic target in AP (24). From this study we found negative correlation (-v) between IL-33 and T3, T4 respectively while there was positive correlation (+v) between IL-33and TSH in control groups, so we found positive correlation (+v) between T3 and TSH with IL-33 when compared between them ,but we show a negative correlation (-v) between IL-33 and T4 when compared between patients group . No study found to agreement or disagreement with this study to compared between them, but other study also found negative correlation (-v) between IL-6 -and IL-10 positively correlated with serum T3 and T4 but not with serum TSH. Also, a highly Positive correlation was found between IL-6 and 1L-10 in the hyperthyroid groups (25)..From the present study, a conclusion can be stated that IL-33 increase concentration in sera of hyperthyroidism female of Iraqi patients. This is due to interleukins that regulate information transfer among different types of leukocytes during various stages of immune or inflammatory response, this is confirmed by increase T3, and T4 but decrease in TSH concentration. This finding may indicate that autoimmune disease like hyperthyroidism might influence cytokine production in these patients

References:

1- Dario M. T., Geoffrey C. L., Jerome V.R. : Kochar's Clinical Medicine for Students: Lippincott Williams & Wilkins; Fifth edition(2008) . Pp.402-403

2 - Adelmanne ,G.A. (2011):Heart Faliure .j.Cardiology Essential Clinicl Practice edited by Springer Verlage London Limited p.137

3- Kittisupamongkol W. : Hyperthyroidism or thyrotoxicosis? Cleve Clin J. Med. (2009); 76(3):152.

4 – Hyperthyroidism. The American Thyroid Association. http://www.Thyroid.org/what-is-hyperthyroidism. Accessed July 17, 2012

5- Walter Siegenthaler: Differential Diagnosis in Internal Medicine: From Symptom to Diagnosis, (2007): p.485: Thieme.

6-Janet E. H., Lynnette K. N. : Handbook of diagnostic endocrinology:(2003) pp. 124-125

7- Web MD better information .Better healt ©2005-2012 WebMD, LLC. All rights reserved.WebMD does not provide medical advice, diagnosis or treatment.

8- Interleukin (IL) (protein) – Britannica Online Encyclopedia (2013)

9- National Cancer Institute at the national institute of health from online 1-800-4-cancer .Live help online chat.

10-Ashley M Miller : Role of IL-33 in inflammation and disease Journal of Inflammation (2011) 8:22

11- Menachem B. Zidon O, Avital A,et,al : "Astrocytes support hippocampal-dependent memory and long-term potentiation via interleukin-1 signaling". Brain Behav. Immun. (2011) 25 (5): 1008

12- From Wikipedia, the free encyclopedia 2012. Cytokines &Cells online pathfinder Encyclopedia. 2012

13- Arroyo-Villa I, Bautista-Caro MB Balsa A, et al : Frequency of Th17 CD4+ T cells in early rheumatoid arthritis: a marker of anti-CCP seropositivity., Arthritis Res Ther . (2012) 7(8):e42189.

14-Eggleton P., Bremer E., Tarr JM, et al: Frequency of Th17 CD20+ cells in the peripheral blood of rheumatoid arthritis patients are higher compared to healthy subjects. Arthritis Res Ther. (2011) 13(6):R208.

15- Gabay C , McInne BL. : The biological and clinical importance of the 'new generation' cytokines in rheumatic diseases .J. Arthritis Research & Therapy , (2009)11:230.

16-Yuan FL: IL-33: a promising therapeutic target for rheumatoid arthritis? Expert Opin Ther Targets. ; (2011) 15(5):529-34.

17-Gaby P. Dominique T. Celine L.,et al: Inhibition of Interleukin-33 Signaling Attenuates the Severity of Experimental Arthritis. Arthritis and rheumatism; (2009) 60(3): 738–749

18- Reddy P.A., Harinarayan C.V., Sachan A., et al: Bone disease in thyrotoxicosis. Indian J Med Res 135: (2012) pp 277-286

19-Mok MY : Serum levels of IL-33 and soluble ST2 and their association with disease activity in systemic lupus erythematosus. Rheumatology (Oxford) ; (2010) 49(3):520-7.

20-Cevikbas F and Steinhoff M : IL-33: a novel danger signal system in atopic dermatitis. J Invest Dermatol.; (2012)132(5):1326-9.

21-McLaren JE, Michael DR, Salter RC, et.al: IL-33 reduces macrophage foam cell formation. J Immunol. ;(2008) 185(2):1222-9.

22- Kakkar R,and Lee RT(2008) :The IL-33/ST2 pathway: therapeutic target and novel biomarker. Nat Rev Drug Discov. ; 7(10):827-40.

23- Wang S., Ding L, Liu SS, et al (2012): IL-33: A Potential Therapeutic Target in Autoimmune Diseases. J Investig Med. (2012) ; 60(8):1151-6

24-Ouziel R: The ST2 pathway is involved in acute pancreatitis: a translational study in humans and mice. Am J Pathol. ; (2012)180(6):2330-9.

25- Mohaned S.M9:Attenuation of Oxidative Stress and Cytokines in Rabbits with Experimentally Induced Hyperthyroidism by Ethanolic Red Cabbage Extraction eng. & Tech. Journal , 2012 30 (9).