

LOCAL EXPRESSION LEVEL OF IL17 & IL4 CYTOKINES REFLECT THEIR ROLE IN THE PATHOGENESIS OF HASHIMOTO'S THYROIDITIS**^{1*}Ibrahim A. Altamemi, ²Thair Wali Ali, ³Attaa A. Altamimi and ⁴Ahmed Kifah Alwayly**^{1,2}College of Medicine/ Al-Qadisiyah University.³Al-Kindy Teaching Hospital.⁴Al-Shahid Alsadr Hospital.***Corresponding Author: Dr. Ibrahim A. Altamemi**

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Article Received on 05/12/2016

Article Revised on 26/12/2016

Article Accepted on 15/01/2017

ABSTRACT

Hashimoto's thyroiditis, is an autoimmune disease of the thyroid gland characterized by the presence of antibodies directed against the thyroid, and by infiltration of the thyroid gland by lymphocytes. The present study was conducted to investigate the role of IL17 & IL4 cytokines in the pathogenesis of HT in association with disease clinical and pathological feature. Total 46 paraffin embedded section of total thyroidectomy from patients with HT, in addition to 49 with non inflammatory multi-nodular goiter (considered as control group) were included in this study, and collected from Alkindy teaching hospital, all clinical data for each case were collected for each patients, an IHC technique were conducted by using monoclonal antibodies for both Il-17, and Il-4. According to present study the mean cytokine expression in patients with HT was significantly higher than that of control group for both IL-17 and IL-4 ($P < 0.001$, < 0.001 respectively). Moreover, When patients were classified according to T4 serum level into euthyroid and hypothyroid subgroups, the mean IL-17 was significantly higher in euthyroid patients than hypothyroid patients ($P < 0.05$) and it was similar in case of IL-4 ($P < 0.01$). Moreover, there was a significant positive correlation between TSH level and IL17 ($p \leq 0.051$). While, This study did not demonstrate any significant correlation between IL17 both of T3 & T4. In conclusions, the present study demonstrated a prominent role for IL17 in the pathogenesis of disease severity of Hashimoto's thyroiditis especially through its correlation with TSH level and the degree of hypothyroidism which reflect its indirect role in the degenerative and fibrotic change in thyroid tissue.

KEYWORDS: Hashimoto's thyroiditis, IL(Interleukin), multinodular goiter.**1. INTRODUCTION**

Hashimoto's thyroiditis is an organ-specific Autoimmune disease characterized by diffuse goiter with lymphocytic infiltration and the presence of thyroid-specific autoantibodies, Hakaru Hashimoto, who first described the disease in 1912, Today, it is one of the most widespread thyroid disorders and a cause of hypothyroidism in areas of the world where dietary iodine is sufficient. The incidence is 0.3–1.5 cases per 1000 population per year. The prevalence of positive antibody tests in women is greater than 10% and of clinical disease at least 2%. (A kamizu T *et al.*, 2012) and is most prevalent between 45 and 65 years of age (Paknys *et al.*, 2009). Clinically the patients with Hashimoto's thyroiditis are usually asymptomatic and some patients develop goiters with or without hypothyroid (Adims J *et al.*, 2011). The etiology of Hashimoto's thyroiditis is considered to be multifactorial, involving the interplay of various environmental, genetic (HLA), and immunological factors. (Fisher *et al.*, 2000).

T helper type 2 cells lead to an excessive stimulation and production of B cells and plasmatic cells which produce antibodies against thyroid antigens leading to thyroiditis (Aleksandra Pyzik *et al.*, 2015). Th1, and Th2 cells produce interferon- (IFN-) gamma, and interleukin- (IL-) 4, respectively. Nanba *et al.* reported that IFN-gamma and IL-4 gene polymorphisms, which are related to higher IFN-gamma and lower IL-4 production, respectively, are more frequent in patients with severe HT than in those with mild HT (Nanba T. *et al.*, 2012). Also, Bossowski *et al.* demonstrated an elevated level of Th17 cells in children with untreated Hashimoto's disease, which suggests the participation of these cells in the induction and development of the disease. Thus, according to such controversy the aims of the present study is to find out; the proposed role of IL-17 and Il-4 in the immune-pathogenesis of Autoimmune hashimoto's thyroiditis through analysis of their local expression level with patient's T3, T4, and TSH serum hormonal level.

2. MATERIALS AND METHODS

Ninety five (5 male and 90 females) where (46 patients with Hashimoto's thyroiditis and 49 with non inflammatory multinodular goiter), Mean age 37.30 ± 10.47 years for patients versus 35.02 ± 8.78 years for control ($P=0.290$), who were confirmed histopathologically by Dr. Attaa A. Altamimi in Al kindy hospital/ Baghdad. Samples were collected during the period between March 2015 and of April 2016. Information about each patient was obtained from previous medical reports. Paraffin embedded blocks were retrieved along with the histopathological report of each patient from histopathological laboratory. In addition, Adequate thin paraffin embedded sections ($4\mu\text{m}$ thick) of Hashimoto's thyroiditis and multi-nodular goiter were prepared. An Immunohistochemical technique was used to evaluate the local expression level of both IL-17 and IL-4 by using monoclonal Ab from Santa Cuze biotechnology company (USA), and the procedure is done according to manufacturer instructions.

3. RESULTS AND DISCUSSION

3.1. Demographic characteristic of the study

Mean age of patients group was not significantly different from that of control group, 37.30 ± 10.47 years versus 35.02 ± 8.78 years ($P \leq 0.290$). Also, Majority of patients with HT were female, as well as patients with

colloid nodular goiter (control group) and there was no significant difference in distribution of patients according to gender in both groups ($P \geq 1.000$) as shown in table 1, The possible explanation for high female predominance in thyroid autoimmunity might be associated with the X chromosome containing a number of sex and immune-related genes which are of key importance in the preservation of immune tolerance. Increased immunoreactivity might therefore be related to genetic defects of the X chromosome, such as structural abnormalities or monosomy (Tao Yang and Xiaoyun Liu, 2014).

Table 1: Distribution of patients and control subjects according to gender.

Gender	Control n (%)	HT n (%)	χ^2	P-value
Male	3 (6.1)	2 (4.3)	0.000	1.000
Female	46 (93.9)	44 (95.7)		
Total	49 (100.0)	46 (100.0)		

Mean serum of T3 was significantly higher in patients group when compare with control group ($P \leq 0.003$), while mean serum T4 level did not show significant differences between the two groups ($P \geq 0.200$), moreover, mean serum TSH was significantly higher in patients group than in control group ($P \leq 0.030$), table 2.

Table 2: Comparison of mean T3, T4 and TSH between patients and control groups.

Hormone	Control (n = 49)		HT (n = 46)		P-value
	Mean \pm SD	Range	Mean \pm SD	Range	
T3	1.71 ± 0.31	1.22 -2.20	1.99 ± 0.58	0.93 -4.00	0.003
T4	88.10 ± 28.86	6.00 -150.30	84.27 ± 26.88	9.40 -132.00	0.200
TSH	2.08 ± 1.56	0.05 -5.30	4.62 ± 7.93	0.05 -40.40	0.030

3.2. Cytokine local expression level.

Mean local cytokine expression in patients with Hashimoto's thyroiditis was significantly higher than that of control group for both IL-17 and IL-4 ($P < 0.001$,

< 0.001 , respectively), table 3, Figure 1 A and 2 B, Immunohistochemical staining for slide tissue samples of both patients and controls.

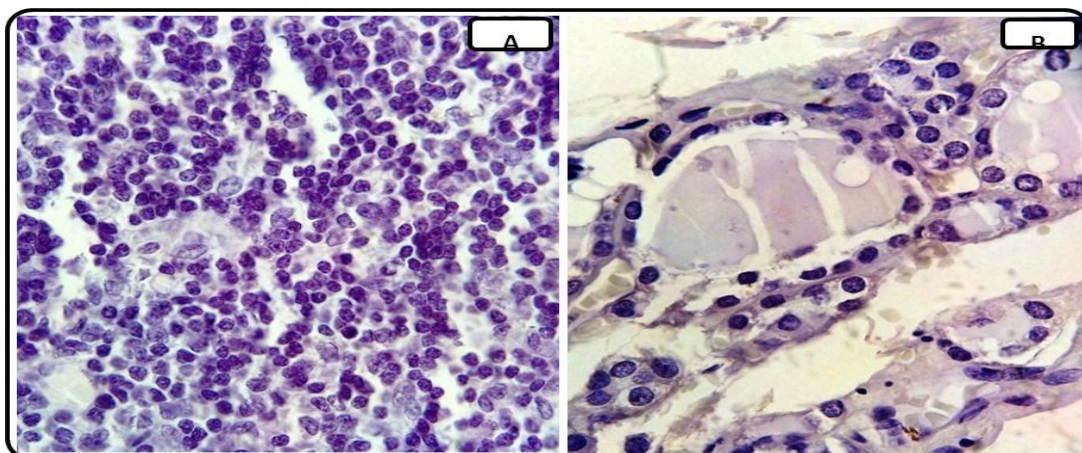


Figure 1: A. Immunohistochemical intracellular staining of IL17 in Hashimoto's thyroiditis section stained by Diaminobenzidine (DAB) (brown stain) counterstained with Myer's hematoxyline (blue stain). (A) IL17 cytoplasmic expression in Hashimoto's thyroiditis section, (B) IL17 cytoplasmic expression in multinodular goiter section, magnification power from A and B (400X).

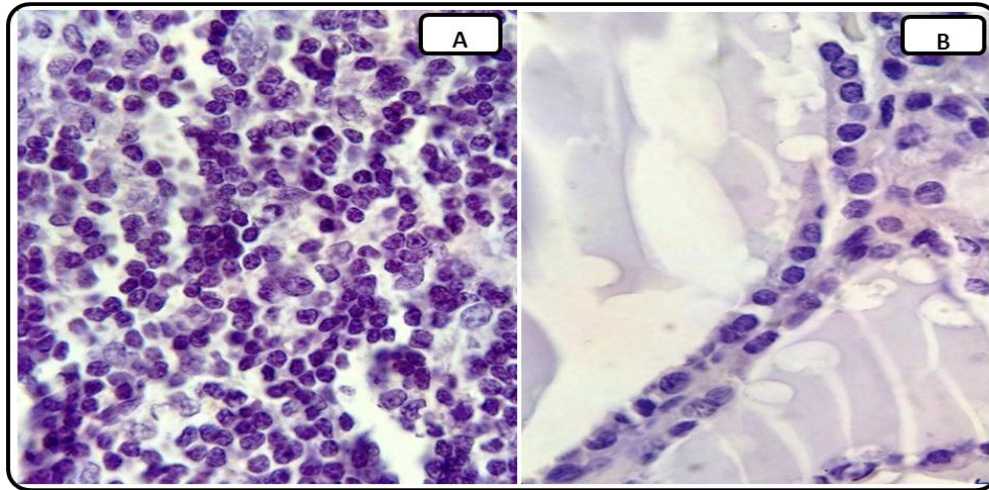


Figure 2: B. Immunohistochemical intracellular staining of IL4 in Hashimoto's thyroiditis section stained by Diaminobenzidine (DAB) (brown stain) counterstained with Myer's hematoxyline (blue stain). (A) IL4 cytoplasmic expression in Hashimoto's thyroiditis section, (B) IL4 cytoplasmic expression in multinodular goiter section, magnification power from A to D (400X).

Table 3: Comparison of mean IL-17 cell and IL-4 cell % between patients and control groups.

Cytokine %	Control (n = 49)		HT (n =46)		P-value
	Mean ±SD	Range	Mean ±SD	Range	
IL17 %	49.01 ±14.76	31.50 -86.25	61.25 ±15.53	33.50 -95.00	<0.001
IL4 %	44.89 ±16.91	27.00 -97.75	65.03 ±17.08	45.00 -92.00	<0.001

Moreover, When patients were classified according to T4 serum level into euthyroid and hypothyroid subgroups, the following results was obtained: mean IL-17 % was significantly higher in euthyroid subgroup than control group (P<0.001), and the same was applied to IL-4%

(P<0.001); Moreover, Mean IL-17 shows significant higher expression in euthyroid patients than hypothyroid patients (P≤ 0.039) and it was similar in case of IL-4% (P<0.01), table 4 and figure 3.

Table 4: Comparison of mean IL-17 and IL-4 % among patients according to thyroid status.

Cytokine %	Control	Euthyroid	Hypothyroid	P1	P2	P3
IL17% (Mean±SD)	49.01±14.76	62.93±15.14	50.08 ±14.54	<0.001	0.892	0.039
IL4 % (Mean ±SD)	44.89±16.91	67.89±16.51	46.00 ±1.18	<0.001	0.255	<0.001

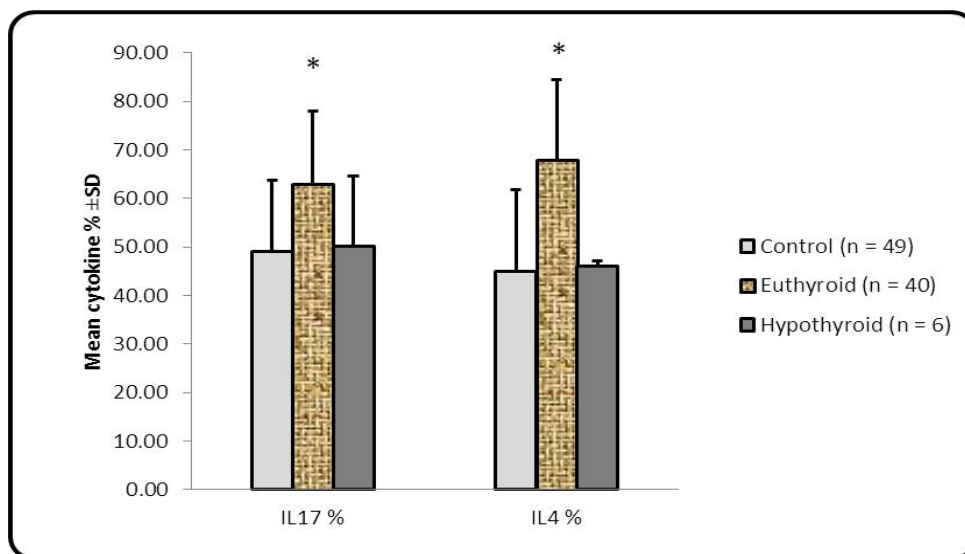


Figure 3: Comparison of mean IL-17 and IL-4 % among patients according to thyroid status.

In the present study, The Th17 and Th2 cytokines status is altered in HT in a way that euthyroid HT is associated with higher IL-17 and IL-4 levels compared to hypothyroid HT and non-thyroiditis controls, The results of this study agree with a study done by (Ceyla Konca Degertekin *et al.*, 2016) since the found that IL-17 levels was higher in euthyroid HT patients compared to controls. These finding suggest that Th17 system might have different functions in different stages of HT. Li *et al.* showed that there was progressive decline in serum IL-17 levels with respect to the degree of hypothyroidism (Li *et al.*,2013). Li *et al.* suggest that the Th17 response might have a pathological role in the earlier stages of HT leading to local inflammation and local stromal fibrosis.

Regarding the IL4 expression level and it's correlation with disease progression, Unfortunately there is no previous study demonstrate the role of such cytokines in the pathogenesis Hashimoto's thyroiditis, Thus, the present study shows a prominent role for IL4 in disease progression in association with IL17 and further studies are needed to clarify at which stage IL4 have more powerful effects. However, other studies indirectly focused on the role of Th1 versus Th2 in HT , Gherardo Mazziotti *et al.* performed a single-cell analysis of the intracellular cytokine expression in peripheral CD4+ and CD8+ lymphocytes from patients with Hashimoto's thyroiditis (HT) to investigate the type-1 T-cell response. HT patients showed a higher number of CD4+IFN-g+, CD4+IL-4+ and CD8+IFN-g+ cells than the control subjects. Analysis the intracellular expression of IFN-g and IL-4 in relation to the thyroid function, They found that the euthyroid patients showed more expression of IL-4 in CD4+ lymphocytes than the control subjects (Gherardo Mazziotti *et al.*, 2003), Which agree with this study. Moreover, the expression of IL-4 in CD4+ cells from hypothyroid patients was significantly lower than that seen in the euthyroid cases and comparable to that found in the control subjects.

3.3. Correlation of IL-17 and IL-4 along with T3, T4, and TSH serum hormonal level.

Correlations between cytokine IL-17 and IL-4 % and serum hormone levels were shown in table 5. Generally speaking, there was no significant correlation.

Table 5: Correlation between local cytokines expression level and serum hormone levels.

Hormone	Correlation	IL17	IL4
T3	R	0.032	0.219
	P	0.830	0.143
T4	R	0.159	0.263
	P	0.291	0.078
TSH	R	0.290	0.201
	P	0.051	0.181

In the above table there is a significant positive correlation between TSH level and IL17 ($P \leq 0.051$),

This results disagree with a study done by (Ceyla Konca Degertekin *et al.*,2016), Since they propose that hypothyroidism itself had a depressive effect on Th17 cytokine responses. The negative correlations of IL-17 with TSH levels in their study might support this inhibitory effect. These findings suggest that Th17 system might have different functions in different stages of HT. in addition; present study did not show significant correlation between IL17 and both of T3 & T4.

Regarding the correlation between IL4 and T4, This study shows a border line correlation, ($P \geq 0.078$), More accurate results may be obtained when more samples used during another study, Also different type of sample may be more informative about the role of this cytokine in the disease process.

In the present study. Table 5, shows the correlation between IL4 and TSH in which there was no significant correlation between them. Keeping in mind this is first study conducted in Iraq to find out correlation between IL4 & HT, Unfortunately, There is no previous study focusing the role of IL4 in the pathogenesis of Hashimoto's thyroiditis. The absent of significant correlation may be due to small sample size.

In conclusions, the present study demonstrated a prominent role for IL17 in the pathogenesis of disease severity of Hashimoto's thyroiditis especially through its correlation with TSH level and the degree of hypothyroidism which reflect its indirect role in the degenerative and fibrotic change in thyroid tissue.

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