

Association between Helicobacter Pylori Infection and Autoimmune Hashimoto's Thyroiditis

NaglaaGamal Abo Elazaim*, Ahmed Mohammed AbdElrahman*, MagdyAbdElkarim AL Matboly*, Ahmad FathyAbdElaziz* andRafikAbdelatefMetwally *

*Department of Clinical Pathology, Faculty of Medicine, Al-Azhar University

Corresponding author: RafikAbdelatefMetwally, Mobile: 00201014193962,

E-Mail: rafikmhmd@gmail.com

Abstract

Background: Autoimmune thyroid diseases are multifactorial conditions that result from genetic predisposition in combination with environmental risk factors. Some bacteria and viruses are suspected of being able to mimic the antigenic profile on the thyroid membrane and play an important role in the onset of auto immune diseases.

Object: The aim of this study was directed to investigate the association between helicobacter pylori infection and Hashimoto's thyroiditis.

Methodology: The present study included 100 subjects divided into two groups: *Patient group*: this group included 70 patients, chosen from attending outpatient medical clinic patients and were newly diagnosed as having Hashimoto's thyroiditis guided by clinical diagnosis and detection of TSH, free T3, free T4, thyroperoxidase antibodies (TPOAbs) and Thyroglobulin antibodies (TgAbs) in serum samples. *Control group*: This group included 30 age- and sex-matched apparently healthy individuals with normal thyroid profile (TSH, freeT3 and freeT4).

Results: In the present work, there was abnormal elevation of TSH in 60 patients (85.7%), compared to just one subject (3.3%) of control group with statistically significant difference between groups. In the present work, there was significant and proportional (positive) correlation between HPIgG and each of TPO and ATG values.

Conclusions: Our results showed significant association between Hashimoto's thyroiditis and HP infection. This association might be related to the disease severity, and might reflect the need of adding anti-HP medications as additional choice in management of Hashimoto's thyroiditis.

Keywords: Helicobacter Pylori, Hashimoto's Thyroiditis

Introduction

Helicobacter pylori is gram-negative, microaerophilic, spiral, and cosmopolitan bacterium. Up to 76-95% of gastric cancers and 90% of duodenal ulcer is associated with *H. pylori* infection⁽¹⁾.

H. pylori infection is nearly 50-75% of the worldwide demography, in the developing countries 70% of people are affected, whereas the percentages 25-50% in the developed countries⁽²⁾. In Egypt *H.pylori* prevalence was studied in a cohort study by the Egyptian ministry of health and the US Naval Medical Research Unit (namru-3) that identified a prevalence of 72.38 of *H.pylori* infection in Egypt. This high prevalence is due to low socioeconomic status and living in rural areas⁽³⁾. In the United States, the annual cost associated with peptic ulcer disease is estimated to be \$6 billion and gastric cancer kills over 700000 people per year globally⁽²⁾.

The prevalence rate of Hashimoto's thyroiditis is 1-4% with incidence of 30-60/100000 population per year. Chronic

autoimmune (Hashimoto's) thyroiditis is the most common and extensively studied organ-specific autoimmune disorder in humans. It is the most common cause of hypothyroidism in iodine-replete areas of the world. However, in clinical practice, the diagnosis of the disease is based on positive serum thyroid autoantibodies mainly thyroperoxidase antibodies (TPOAbs), thyroglobulin antibodies (TgAbs) and the characteristic echo graphic pattern of diffuse or irregular hypoechogenicity of the thyroid gland⁽⁴⁾.

Also there is significant decrease of free T3 and free T4 in HP infection ⁽⁵⁾. Finally *Helicobacter pylori* infection is reportedly associated with extradiagnostic diseases such as immune thrombocytopenic purpura and coronary heart disease. The risk factors for autoimmune thyroid diseases (ATDs) remain largely unknown, and whether *H. pylori* infection is associated with ATDs is still controversial⁽⁶⁾.

Aim of the work

The aim of this study was directed to investigate the association between helicobacter pylori infection and Hashimoto's thyroiditis.

Subjects and methods

This case-control study was carried out at the Outpatient Clinics at AL-Housen University Hospital over the period between November 2016 and September 2018. **The study was approved by the Ethics Board of Al-Azhar University.**

Study Population:

Patient group:

This study included 70 patients, chosen from attending outpatient medical clinic patients were newly diagnosed as having Hashimoto's thyroiditis guided by clinical diagnosis and detection of TSH, free T3, free T4, Thyroperoxidase antibodies (TPOAbs) and Thyroglobulin antibodies (TgAbs) in serum samples. They were 17 males and 53 females with their age ranging between 19 and 63 years.

Inclusion criteria

- Newly diagnosed Hashimoto's thyroiditis patients.
- Patients not receiving specific medications for thyroid illness.

Exclusion criteria:

- Patients received medication for Hashimoto's thyroiditis or helicobacter pylori prior to the study.
- Patients with chronic systemic diseases as chronic renal failure, chronic liver disease and diabetes mellitus.

Control group:

Additional, 30 age- and sex-matched apparently healthy individuals with normal thyroid profile (TSH, freeT3 and freeT4) were included as a control group. They were 11 males and 19 females with their age ranging between 20 and 58 years.

All subjects included in this study were submitted to the following

- I. Full clinical assessment including thorough medical history and clinical examination.
- II. Laboratory assessment for:
 1. Routine investigations: CBS, RBS, ALT, AST, bilirubin, serum creatinine and urea.
 2. Thyroid profile TSH, free T3 and free T4.

3. Thyroid autoantibodies thyroperoxidase antibodies (TPOAbs) and thyroglobulin antibodies (TgAbs).

4. Serum quantitative Helicobacter pylori IgG.

5. Detection of Helicobacter pylori antigen in stool samples by rapid test.

6. Confirmation of Helicobacter pylori antigen in stool positive samples by polymerase chain reaction (PCR).

Samples:

Venous blood samples and stool samples were obtained from all participants (patients and controls) after taking written medical consent for participation in the study.

Sample preparation

Five ml venous blood sample was extracted from all subjects and transferred into plain gel tube, left at room temperature for 20 minutes to clot, then centrifuged at 3000 rpm for 10 minutes. Serum was separated and divided into two aliquots, one used for TSH, FT3, FT4, TPOAbs and TgAbs on the same day of collection, and the second aliquots was kept frozen at -20 °C for H.pylori IgG estimation by ELISA.

Stool samples obtained and examined by *one step test* for the detection of *H pylori* Ag in the same day of collection. If proved positive it kept frozen at -20°C for confirmation by molecular diagnosis (PCR).

Methods"

- 1 –Detection of TSH, free T3 and free T4.

- 2- Detection of anti-thyroglobulin antibodies (Anti-Tg).

- 3-Detection of antithyroid-specific peroxidase (Anti-TPO).

- 4-Detection of helicobacter pylori IgG in serum by quantitative (EIA).

- 5-Detection of *H pylori* Ag in stool sample by one step test.

- 6- Detection of *H pylori* Ag in stool sample by polymerase chain reaction (PCR).

DNA Extraction:

The protocol of DNA extraction was carried on according to PSP spin stool DNA kit (Invitex, Germany) which is optimized to extract DNA from stool samples.

The RTA spin filter was transferred in a new RTA receiver tube, 500 µl wash buffer I was pipetted onto the RTA-spin filter, centrifuged at 10.000 rpm for 1 min, and then the flow-through and the RTA receiver tube were discarded.

The RTA spin filter then was placed in a new RTA receiver tube, 700 µl wash buffer II were pipetted onto the RTA spin filter and centrifuged at 10.000 rpm for 1 min and the flow-through was discarded (to eliminate any traces of ethanol, the RTA receiver tube was centrifuged again for 3 min at maximum speed, then discarded).

The RTA spin filter was transferred into a new 1.5 ml receiver tube and 100-200 µl of elution buffer D (preheated to 70°C) was pipetted directly onto the center of the membrane of the RTA spin filter and incubated for 1 min at RT and then centrifuged at 10.000 rpm for 1 min.

The RTA-spin filter was discarded and the eluted DNA was stored immediately at -20°C.

Determination of concentration, yield, and purity of DNA:

- DNA yields were determined from the concentration of DNA in the eluate, measured by absorbance at 260 nm.
- Purity was determined by calculating the ratio of absorbance at 260 nm to absorbance at 280 nm. Pure DNA has an A260/A280 ratio of 1.7-1.9.

DNA amplification:

Materials:

1. Template DNA.
2. Dream Taq Green PCR master mix supplied by (Fermentas, USA) that is composed of (DreamTaq™ DNA polymerase, optimized DreamTaq™ Green buffer, MgCl₂ and dNTPs.).
3. Primers:
For the 16 s rRNA gene (399 bp)

Two primers were supplied as lyophilized powder to be reconstituted upon use.

- *Heli f*: (5'-AAC GAT GAA GCT TCT AGC TTG CTA G3')
 - *Helir*: (5'GTG CTT ATT CAT CAG ATA CCG TCA T3')
- For the *glmM* gene (294 bp).

- F: 5-
GGATAAGCTTTTAGGGGTGTT
AGGGG-3.
- R: 5-
GCTTACTTTCTAACACTAACGC
GC-3.

4. Water, nuclease-free.
5. Negative control: sterile nuclease free water (injection water).

6. Positive control: *E.coli* VC167 supplied by (NAMRU3 lab, Egypt).

Procedure:

For the 16 s r RNA

An initial denaturation at 94°C for 5 min and 35 cycles of 94°C for 30 s, 56°C for 1 min and 72°C for 1 min. This was followed by a final extension of 72°C for 10 min with initial delay for 1 minute. Amplified products were stored at -20°C.

For the *glmM* gene (294 bp)

One cycle of denaturation at 94°C × 5 min; 35 cycles at 94°C × 1 min, annealing at 56°C × 1 min, and elongation at 72°C × 2 min, followed by a final elongation step by 1 cycle at 72°C × 7 min. 15°C.

Sensitivity of the primers was determined by testing other bacterial strains from related genus e.g. (*E.coli*).

Detection of the amplification products:

Agarose gel electrophoresis was used to separate molecules. It is based on charge, size and shape of the molecules.

Interpretation:

- The gel was examined under ultraviolet light as ethidium bromide intercalates between the bases of the DNA which fluoresces. Photographs were taken using a Polaroid camera which is connected to computer system.
- Marker gave different bands ranging from 100-2000 bp.
- The negative control was examined to exclude any source of contamination.
- Samples were compared with positive controls.
- Positive samples give band at 450 bp position.

Statistical methods:

Data were statistically described in terms of mean ± standard deviation (± SD), median and range, or frequencies (number of cases) and percentages when appropriate. Comparison of numerical variables between the study groups was done using Student *t* test for independent samples. For comparing categorical data, Chi square (χ^2) test was performed. Exact test was used instead when the expected frequency is less than 5. *p* values less than 0.05 was considered statistically significant. All statistical calculations were done using computer programs SPSS (Statistical Package

for the Social Science; SPSS Inc., Chicago, IL, USA) version 15 for Microsoft Windows.

Results

The present study included 100 subjects divided into two groups; patient group (70 patients) and control group (30 normal subjects).

Table (1): Age distribution among studied populations.

	Mean	SD	t	p
Patients	36.70	3.73	0.57	0.57 (ns)
Control	37.96	13.93		
Total	37.08	13.06		

In the present study, patient age ranged from 19 to 63 years, and there was no significant difference between study and control groups (36.70±9.73 vs 37.96±10.93 years respectively).

Table (2): Sex distribution among studied populations.

		Group				Total	
		Patients		Control			
		n	%	n	%	n	%
Sex	Male	1	24.3	1	36.7	2	28.0
		7	%	1	%	8	%
	Female	5	75.7	1	63.3	7	72.0
		3	%	9	%	2	%
Statistics		X ² = 1.59, p = 0.21(ns)					

In the present study, females represented 72% of all studied populations and there was no significant difference between study and control groups, as females represented 75.7% of study group compared to 63.3% of the control group.

Table (3): Normal and abnormally elevated TSH in studied groups.

	Group				Total	
	Patients		Control			
	n	%	n	%	n	%
Normal (0.27- 4.6)	1	14.3	2	96.7	3	39.0
	0	%	9	%	9	%
Abnormal y elevated	6	85.7	1	3.3%	6	61.0
	0	%			1	%
Statistics	$X^2 = 59.90 , p < 0.001 *$					

In study group, there was abnormal elevation of TSH in 60 patients (85.7%), compared to just one subject (3.3%) of control group with statistically significant difference between groups.

Table (4): Normal and abnormal free T3 in studied populations.

		Group				Total	
		Patients		Control			
		n	%	n	%	n	%
FT 3	Normal	5	78.6	2	76.7	7	78.0
		5	%	3	%	8	%
	Decreased	7	10.0	0	0.0	7	7.0
			%		%		%
	Increased	8	11.4	7	23.3	15	15.0
			%		%	5	%
Statistics		$X^2=6.83, p=0.033^*$					

In the present work, there was 10% of subjects in study group who had decreased values of FT3 and 11.4% had increased levels of FT3. This was compared to 0.0% and 23.3% in the control group respectively. There was significant difference between groups.

Table (5): Normal and abnormal free T4 in studied populations.

		Group				Total	
		Patient s		Control			
		n	%	n	%	n	%
Free T4	Normal	4	64.	2	96.	7	74.
		5	3%	9	7%	4	0%
	Decreased	2	28.	0	.0	2	20.
		0	6%		%	0	0%
	Increased	5	7.1	1	3.3	6	6.0
			%		%		%
Statistics		$X^2=12.05, p=0.002^*$					

Decreased values of FT4 was reported in 28.6% of study group compared to none in control group, while increased values of FT4 was reported in 7.1% in the study compared to 3.3% of the control group with significant difference between both groups.

Table (6): Normal and abnormal TPO in studied populations.

		Group				Total	
		Patients		Control			
		n	%	n	%	n	%
TPO	Normal	1	22.9	3	100.0	4	46.0
		6	%	0	%	6	%
	Increasing	5	77.1	0	0.0%	5	54.0
		4	%			4	%
Statistics		$X^2 = 50.31, p < 0.001^*$					

In the present work, there was statistically significant increase of abnormally increased values of TPO in the study when compared to control group (77.1% vs 0.0% respectively).

Table (7): Normal and abnormal ATG in studied populations.

		Group				Total	
		Patients		Control			
		n	%	n	%	n	%
ATG	Normal	1	17.1	3	100.0	4	42.0
		2	%	0	%	2	%
	Increased	5	82.9	0	0.0%	5	58.0
		8	%			8	%
Statistics		$X^2 = 59.18, p < 0.001^*$					

In the present study, there was statistically significant increase of abnormally increased values of ATG in study when compared to control group (82.9% vs 0.0% respectively).

Table (8): HPIgG among studied populations

	Patients	Control	t	p
Mean	39.09	21.40	3.40	0.001*
5% Trimmed Mean	37.42	20.09		
Median	32.50	12.00		
Variance	619.76	440.46		
Std. Deviation	24.89	20.99		
Minimum	10.00	1.00		
Maximum	105.00	67.00		
Range	95.00	66.00		

In the present study, HPIgG ranged from 10 to 105 in study group and from 1 to 67 in control group, and there was significant increase in study when compared to control group (39.09 ± 24.89 vs 21.40 ± 20.9 respectively).

Table (9): Results of H. pylori antigen in stool among studied populations.

		Group				Total	
		Patients		Control			
		n	%	n	%	n	%
H.pylori antigen in stool	Positive	3	54.3	6	20.0	4	44.0
		8	%		%	4	%
	Negative	3	45.7	2	80.0	5	56.0
		2	%	4	%	6	%
Statistics		$X^2 = 10.01, p = 0.002^*$					

As regard to H. pylori antigen in the stool, it was positive in 38 subjects (54.3%) in the study group, compared to 6 subjects (20%) of the control group, and there was significant increase in study when compared to control groups.

Table (10): Results of H. pylori after PCR examination for positive cases in stool test among studied populations.

		Group				Total	
		Patients		Control			
		n	%	n	%	n	%
HP by PCR	Positive	2	41.4	3	10.0	3	32.0
		9	%		%	2	%
	Negative	4	58.6	2	90.0	6	68.0
		1	%	7	%	8	%
Statistics		$X^2 = 9.53, P = 0.002^*$					

By PCR, the total positive cases in study group were 29 (41.4%), compared to 3 cases (10.0%) in the control group, with significant difference between study and control group.

Table (11): Association between H. pylori (PCR) and other studied parameters in study group.

	Positive		Negative		t	p
	Mean	S.D	Mean	S.D		
Age	37.17	9.86	36.37	9.75	0.33	0.73
TS	24.78	2.35	37.71	2.75	1.37	0.17
FT3	3.17	0.05	3.55	0.20	1.38	0.17
FT4	0.77	0.069	1.43	0.045	1.02	0.30
TPO	643.29	38.45	171.53	25.99	6.02	<0.001*
ATG	411.57	3.92	41.01	3.31	5.60	<0.001*
HP IgG	56.48	4.25	26.78	1.82	6.05	<0.001*

In the present work, when comparing positive and negative H.pylori subjects as detected by PCR, there was significant increase of TPO, ATG and HP IgG in positive when compared to negative subjects. However, no significant difference was found as regard to age, TSH, free T3 and free T4.

Table (12): Correlation between HP IgG and other studied variables in study group.

	HP IgG	
	r	p
Age	0.17	0.15
TSH	-0.22	0.06
FT3	-0.15	0.20
FT4	0.01	0.96
TPO	0.23	0.049*
ATG	0.29	0.015*

In the present work, there was mild, significant and proportional (positive) correlation between HP IgG and each of TPO and ATG values. Otherwise, no other significant correlation was found.

Discussion

H. pylori is increasingly being associated with extragastric diseases. *H. pylori* is widely accepted as a cause of iron deficiency anemia and idiopathic thrombocytopenia, but the jury is still out on other diseases ⁽⁷⁾.

Hashimoto's thyroiditis is considered the most widespread autoimmune endocrine disorder and the most common cause of hypothyroidism. The prevalence rate of Hashimoto's thyroiditis is 1–4% with incidence of 30–60/100000 population per year. The classic form of HT is associated with hypothyroidism, euthyroidism or occasionally hyperthyroidism ⁽⁸⁾.

The present work aimed to investigate the association between helicobacter pylori infection and Hashimoto's thyroiditis.

Our results showed that, the age range was 19 - 63; it means that the autoimmune thyroid disease mostly occurs in adult age. This fact also confirmed by **Akamizuet al.**⁽⁹⁾ who argues that thyroid diseases occurs especially from 20 to 55 during the middle decades but may be seen in any age group, including children.

In the present study, Females represented 75.7% of study group nearly the same results by **Hamid**⁽¹⁰⁾, where 82% of Hashimoto's thyroiditis patients were females because sex hormones may play a role in this aspect, as described in several previous studies. Sex hormones, like estrogen, progesterone, and testosterone are believed to mediate the immune response to many of the sex – based differences in the prevalence of autoimmune diseases⁽¹¹⁾. The sex hormone directly interacts with immune system cells via the receptor on or inside immune cells. Steroid hormones are known by their influence in antibody production and immune cell proliferation ⁽¹²⁾.

In the present work, there was no significant difference between study and control groups as regard to CBC findings, liver and renal function tests .

In study group, there was abnormal elevation of TSH in 60 patients (85.7%),

compared to just one subject (3.3%) of control group with statistically significant difference between groups.

In the present work, there was 10% of subjects in study group who had decreased values of FT3 and 11.4% had increased levels of FT3. This was compared to 0.0% and 23.3% in the control group respectively. There was significant difference between groups. Decreased values of FT4 was reported in 28.6% of study group compared to none in control group, while increased values of FT4 was reported in 7.1% in the study compared to 3.3% of the control group with significant difference between both groups.

Thyroid TPO and ATG antibodies still retain its strong value in the screening for thyroid autoimmunity. There was statistically significant increase of abnormally increased values of TPO in the study when compared to control group (77.1% vs 0.0% respectively). There was statistically significant increase of abnormally increased values of ATG in study when compared to control group (82.9% vs 0.0% respectively).

In the present work, when comparing positive and negative *H.pylori*- subjects as detected by PCR, there was significant increase of TPO, ATG and HP IgG in positive when compared to negative subjects.

In the present work, there was significant and proportional (positive) correlation between HP IgG and each of TPO and ATG values.

Our results showed significant association between Hashimoto's thyroiditis and HP infection. This association might be related to the disease severity, and might reflect the need of adding anti-HP medications as additional choice in management of Hashimoto's thyroiditis.

The current study could not explain the mechanism by which *H. pylori* can evoke Hashimoto's thyroiditis. **Bogdanos and Vergani**⁽¹³⁾ agree with the result of the present study in that “the exact mechanisms by which exposure to a microbe elicits more than one autoimmune manifestation are not well defined” but cross reactive responses against a microbial mimic and several self-antigens have been documented.

Our results are consistent with most of previous studies such as results showed by **Houet al.**⁽¹⁴⁾. Their study presented a systematic

meta-analysis of the correlation between *Helicobacter pylori* (*H. pylori*) infection and autoimmune thyroid diseases (AITD). Fifteen articles including 3,046 cases were selected (1,716 observational and 1,330 control cases). These data were analyzed using Stata12.0 meta-analysis software. They found *H. pylori* infection was positively correlated with the occurrence of AITD (OR = 2.25, 95% CI: 1.72–2.93). Infection with *H. pylori* strains positive for the cytotoxin-associated gene A (CagA) were positively correlated with AITD (OR = 1.99, 95% CI: 1.07–3.70). There was no significant difference between infections detected using enzyme-linked immunosorbent assay (ELISA) and other methods ($\chi^2 = 2.151$, $p = 0.143$). Patients with Grave's disease (GD) and Hashimoto's thyroiditis (HT) were more susceptible to *H. pylori* infection (GD: OR = 2.78, 95% CI: 1.68–4.61; HT: OR = 2.16, 95% CI: 1.44–3.23), while the rate of *H. pylori* infection did not differ between GD and HT ($\chi^2 = 3.113$, $p = 0.078$). They concluded that *H. pylori* infection was correlated with GD and HT, and the eradication of *H. pylori* infection could reduce thyroid autoantibodies.

In conclusion our study revealed that patients with positive TG & TPO antibodies, showed (+ve) *H. pylori* Ab, with significant high titre in their sera. The patients with positive *H. Pylori* Ab showed high serum titre of TG-Ab. In this study *H. pylori*-Ab correlated to thyroid function tests and thyroid antibodies.

The same result were confirmed by **Arslanet *al.*⁽¹⁵⁾**, who reported the presence of a link between *H. pylori* infection and thyroid disorders, including autoimmune thyroid disorders such Hashimoto's thyroiditis.

Conclusions

Our results showed significant association between Hashimoto's thyroiditis and HP infection. This association might be related to the disease severity, and might reflect the need of adding anti-HP medications as additional choice in management of Hashimoto's thyroiditis.

Recommendations

- It is recommended that patients with helicobacter pylori infection should be followed up regularly by repeated tests of TG-Ab and TPO-Ab autoantibodies in their sera, for earlier detection of subclinical autoimmune

thyroid dysfunction before any changes in TSH values might occur.

- It is recommended also to consider that patients with autoimmune thyroid dysfunction should be followed up for detection of *H. pylori* infection even without dyspeptic symptoms because there is positive correlation between *H. pylori* infection and autoimmune thyroid disease especially that on high doses of thyroxin.

References

1. **Ramesh R, Sheng LW, Jing Liet *al.* (2017):** Helicobacter pylori infection: A recent approach to diagnosis and management. *J. Biomed.*, 2: 45-56.
2. **Safavi M, Sabourian R and Foroumadi A (2016):** Treatment of Helicobacter pylori infection: Current and future insights. *W. J.Cli.Cases*, 4: 5-19.
3. **Mohammad MA, Hussein L, Coward A *et al.* (2008):** Prevalence of Helicobacter pylori infection among Egyptian children: impact of social background and effect on growth. *Public Health Nutr.*, 11: 230-236.
4. **Wagner AP (2017):** Vibratory stimulation of thyroid epithelial mimics hormonal stimulation. *Iowa Research Online*, 56:72.
5. **Korani M, Elshayeb E and Sonbal A (2016):** Helicobacter Pylori Infection: Association with Hashimoto's Thyroiditis. *GastroenterolHepatol.*, 4: 11-1.
6. **Yun MC, Tae YK, Eui YK *et al.* (2017):** Helicobacter pylori Association between thyroid autoimmunity and Helicobacter pylori infection. *Korean J. Intern Med.*, 32:309-313.
7. **Testerman TL and Morris J (2014):** Beyond the stomach: An updated view of Helicobacter pylori pathogenesis, diagnosis, and treatment. *World J.Gastroenterol.*, 20: 12781-12808.
8. **Shmueli H, Shimon I and Gitter L (2016):** Helicobacter pylori infection in women with Hashimoto thyroiditis: A case-control study. *Medicine (Baltimore)*, 95:40-74.
9. **Akamizu T, Amino N and DeGrootL (2013):** Hashimoto's thyroiditis. *Endotext.*, 20:12-15.
10. **Hamid ZA (2017):** The possible role of helicobacter pylori infection in hashimoto's thyroiditis. *Fac.Med.Baghdad*, 59:79-82.

11. **Zandman-Goddard G, Peeva E and Shoenfeld Y (2007):** Gender and autoimmunity. *Autoimmunity Reviews*, 6:366-372.
12. **Fairweather D, Frisancho-Kiss S and Rose N(2008):** Differences in autoimmune disease from a pathological perspective. *Am. J. Pathol.*, 173: 600-609.
13. **Bogdanos D and Vergani D(2006):** Origin of cross-19 reactive autoimmunity in primary biliary cirrhosis. *Liver Int. J.*, 26: 633-635.
14. **Hou Y, Sun W, Zhang C et al. (2017):** Meta-analysis of the correlation between Helicobacter pylori infection and autoimmune thyroid diseases. *Oncotarget*, 8(70): 115691–115700.
15. **Arslan MS, Ekiz F, Deveci M et al. (2015):** The relationship between cytotoxin-associated gene A positive Helicobacter pylori infection and autoimmune thyroid disease. *Endocr. Res.*, 40: 211-214.