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Original Article

Impact of *Helicobacter pylori* Virulence on Development of Autoimmune Thyroid Diseases

Hussein Alaa aldeen Saqi, Mohammed Yawoz Nooruldeen[1], Najdat Ali Al-kadhi[1]

Kirkuk General Hospital, Kirkuk Directorate of Health, [1]Medical Laboratory Techniques Department / College of Health and Medical Techniques, Kirkuk/Northern Technical University, Iraq

Abstract

Background: *H. pylori* infection has been linked in some controversial studies to the existence of extra-gastrointestinal disorders like autoimmune thyroid disease (AITD). **Objective:** The study aimed to investigate the impact of the *H.pylori* virulence factor on progression of autoimmune thyroid disease. **Materials and Methods:** The study carried out on 90 patients (male and female) with thyroid disorders, and 50 healthy individuals as a control group. Venous blood samples were drawn from all participants, serum obtained after centrifugation and kept in in a labeled Eppendorf tube then stored at -20°C till used in biochemical and immunological assays including: estimation of anti *H.pylori* IgG to investigate *H.pylori* infection and detection anti-cag-A IgG titer by ELISA method, while measurement of (T3), (T4), (TSH) and (ATPO) by (e601 cobas) automated immunoassay analyzer. **Results:** The results of the study recorded a significant increase in mean of TSH concentration ($P < 0.01$) in patients group particularly in hypothyroidism patients, and a significant increase recorded ($P < 0.01$) in patients according ATPO level in comparison to control group. On other hand the highest prevalence of *H.pylori* IgG positive (84%) occur in autoimmune thyroid diseases (AITD) group followed by (63%) for non-AITD while (56%) occur in control group. The results also recorded a highly significant increase in titer of *H.pylori* IgG ($P < 0.01$) in patient group as compared to control group, the proportion of female was significantly higher in AITD group (75%), however, there was a significant increase ($P < 0.01$) in the level of anti-cag IgG in patients as compared to control group, The ratio of infection with *H.pylori* was greater in the Hashimoto's thyroiditis group (85.2%) than in the Graves' disease group (82.4%). **Conclusion:** our findings state that H. pylori by expressing Cag A may have an impact on the development of autoimmunity thyroiditis.

Keyword: Autoimmune thyroid diseases, Helicobacter pylori, virulence

Introduction

Helicobacter pylori (*H. pylori*) is the most common bacterial infectious agent in the world.[1,2] Infection with Helicobacter pylori is linked to the occurrence and progression of chronic gastritis, peptic ulcer, gastric cancer, and other gastrointestinal disorders.[3,4]

Helicobacter pylori is a spiral-shaped, gram-negative bacterium with lophotrichous flagella that measures 2 to 4 µm in length and 0.5 to 1 µm in width.[5,6] The bacterium is a slow-growing microaerophilic organism, *H.pylori* infection occurs throughout the world, but the prevalence varies greatly between regions and between populations within the same country.[7] It was believed that if *H.pylori* was acquired and left untreated, infection would persist throughout life. However, there is evidence that *H.pylori* acquisition occurs primarily in early childhood.[8,9]

The pathogenesis of *H.pylori* occur in three processes of colonization, immunological evasion, and disease induction.[10] It contains several virulence genes, including cytotoxin associated antigen (CagA) and cytotoxin associated gene E (CagE) genes within the cag pathogenicity island, Such genes code for proteins associated with bacterial pathogenicity.[11]

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Address for correspondence: Mr. Hussein Alaa aldeen Saqi, Kirkuk general hospital, Kirkuk directorate of health, Iraq. E-mail: Husseyin.saki@gmail.com

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The presence of the cytotoxin-associated gene A (CagA) is one of the most important virulence determinants of *H.pylori* infection, and it may be used to identify the most virulent strains.[12]

Autoimmune thyroid disease (AITD) is One of the most frequent organ specific autoimmune disorders, AITD has multiple pathogenesis, including both genetic and environmental variables, and is a risk factor for thyroid malfunction.[13,14] Autoimmune thyroid diseases (AITDs) such as Graves' disease (GD) and Hashimoto thyroiditis (HT) are characterized by reactivity to self-thyroid antigens due to auto reactive lymphocytes escaping tolerance. The pathogenesis of Hashimoto thyroiditis (HT) is mostly caused by cell-mediated autoimmune disease, whereas GD is caused by humoral autoimmunity.[15]

Infectious pathogens such as *Helicobacter pylori*, which can induce chronic inflammation and autoimmune reaction in susceptible individuals, are one of the possible environmental causes of AITDs.[16]

The link between *H.pylori* infection and thyroid disorders has mostly been targeted on thyroid autoimmunity, including studies of a correlation between *H.pylori* infection and thyroid autoimmune diseases such Graves' disease (GD) and Hashimoto thyroiditis (HT).[17,18]

According to Figura*, et al.*, *H.pylori* infection, especially when caused by organisms that express CagA, is intimately associated to GD and HT, were CagA becoming the major factor responsible for elevated thyroid autoantibodies and inflammatory cytokines.[19] So one point remaining obscure that whereas cag A expression by infecting organisms might play an important role in the development or progression of AITDs, so we focused in this study for clarifying the role of *H.pylori* virulence factor in the development or progression of auto immune thyroid diseases.

Materials and Methods

The study was carried out on 140 male and female subjects including: 90 patients (68 female 75.7%) and (22 male 24.3%) with age group (30- 60) years, suffering from thyroid disorders and 50 healthy people including 14 male and 36 female, as a control group. Patients groups were confirmed by specialist physician in many doctors' private clinic in Kirkuk city from 20th of December 2021 to 15th of March 2022.

An interview was carried out with these patients using questionnaire form designed by the investigator including their name, age, and clinical history. Five ml of venous blood sample collected from all participants allowed to clot at room temperature then centrifuged at 3000 rpm, the serum were kept in a labeled Eppendorf tube then stored in deep freeze -20°C in order to use for biochemical and immunological tests which included: quantitative measurement of Anti – *H.pylori* IgG Antibody titer by Enzyme-linked immunosorbent assay (ELISA) method using (Accu Bind monobind Inc. Kit, USA). Estimation the titer of Human Helicobacter pylori cytotoxin-associated gene A protein IgG(HP-CagA-IgG) using (Sunlong Biotech Co., China.), assays were performed according to the manufacture's protocols.

Measurement the levels of Tri-iodothyronine (T3), tetra iodothyroixine (T4), Thyroid Stimulating Hormone (TSH) and anti-thyroid peroxidase antibody (ATPO) in serum for investigation of autoimmune thyroid diseases, which were assayed by the **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on (Roche Cobas 6000-e601; Roche Diagnostics, Germany) automated immunoassay analyzers according to the manufacturer's instructions.

A total 90 subjects with thyroid disorders in the study, were grouped into two categories: hypothyroidism and hyperthyroidism according to the normal values mentioned in instruction of TSH, T4, and T3 cobas e601 kits, on the other hand the control group (50 subjects) without thyroid diseases (Euthyroid). Results of anti *H.pylori* IgG concentration of more than 20 IU/L confirmed the presence of IgG antibody according to the cut-off point as mentioned in the manufacturer's instructions, and the titer of anti- cytotoxin associated gene IgG (Anti-cag-A IgG) was measured in pg/ml unit. Thyroid autoimmunity was assessed by anti-TPO level according to the manufacture instructions of the kit. A level of more than 34 IU/L is considered as thyroiditis caused by autoimmunity, which is either Graves' disease (GD) in the case of hyperthyroidism or Hashimoto's thyroiditis (HT) in the case of hypothyroidism.

Statistical analysis

Computerized statistical analysis was performed using SPSS statistic program v29 and Prism Graphpad. Comparison was carried out using one way ANOVA T-Test probability (P value). The P value <0.05 was considered statistically significant, The P value <0.01 was considered statistically highly significant and P value >0.01 considered non-significant statistically. Post-HOC test (meaning after this, also called Duncan's Test) also performed to measure the significance between patient groups with each other's not only with control.

Ethical approval

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patients verbal and analytical approval before sample was taken. The study protocol and the subject information and consent form were reviewed and approved by a local ethics committee according to the document number (42471 in 9/12/2021) to get this approval.

Table 1: Concentration of thyroid hormones according study groups

Groups	No	Gender	TSH(μIU/mL)	T4 (nmol/L)	T3 (nmol/L)
			Mean ± S.D	Mean ± S.D	Mean ± S.D
patient groups	22	Male	23.12 ± 4.49 a	103.40 ± 60.3 a	1.802 ± 1.36 a
	68	Female	20.32 ± 3.19 a	110.20 ± 71.74 a	2.218 ± 1.78 a
control	14	Male	2.12 ± 0.77 b	110.72 ± 26.45 a	2.03 ± 0.45 a
	36	Female	2.05 ± 0.91 b	111.19 ± 21.80 a	1.90 ± 0.38 a
P. value			< 0.01	> 0.05	> 0.05

a, b: small letters refers to present significance differences (P < 0.01) between groups at the vertical column.

P ≤ 0.01 = highly significant, p ≤ 0.05= significant, p> 0.05= non-significant

Results

[Table 1] revealed results of the study according to thyroid hormones were recorded a highly significant increase in TSH concentration ($P < 0.01$) in patients group by Mean ± S.D (23.12 ± 4.49) for male and (20.32 ± 3.19) for female suffering from thyroid disorders when compared to control groups males (2.12 ± 0.77) and females (2.05 ± 0.91). While T3, T4 hormones were recorded non-significant (>0.05) between patients and control groups.

From statistical analysis of data according to ATPO level, a significant increase ($P < 0.05$) in the patient group, mean was (44.60 ± 5.80) and (59.77 ± 5.50) for male and female respectively, whereas in the control group it was (8.40 ± 5.09) and (8.86 ± 6.81) for male and female respectively, which is shown in [Table 2].

From a total 90 patients with thyroid disorder, the AITD patients were grouped into 27 (61.4%) patients with Hashimoto's thyroiditis (H.T) and 17 (38.6%) patients with Graves' disease (G.D). The most of the AITD patients were female 77% while male were 23%.

[Table 3] showed the results of the study were documented a highly significant increase ($P < 0.01$) in mean of titer of *H.pylori* IgG in patient group for both male (92.10 ± 17.5) and female (92.57 ± 11.41) as compared to control group.

The virulence of *H.pylori* titer revealed in [Table 4], there was a significant increase ($P < 0.01$) in the mean of anti-cag IgG level in both patient group when compared to control group. In AITD male, the mean was (25.45 ± 6.84) and female was (26.25 ± 7.24) while in control group was (15.59 ± 4.69) in male whereas in female was (17.25 ± 5.07).

[Table 5] shows the prevalence of anti *H.pylori* IgG among autoimmune thyroid disease (AITD), non-AITD and control group. The highest percentage of anti *H.pylori* IgG was detected within autoimmune thyroid disease (AITD) patients (84%), while (63%) among non-AITD individuals and the lowest percentage recorded in the control group (56%) which are summarized in [Figure 1].

from a total of 44 AITD patients, the positive *H. pylori IgG* rate was (85.2%) in Hashimoto's thyroiditis patients, while the rate was (82.4%) in Graves' disease, So the infection rate with *H.pylori* was higher in the (H.T) group than in the (G.D) group, which was (26%) in male and (74%) in female of the H.T group, while in the G.D group having *H.pylori* IgG positive, the male rate was (14.3%) and the female rate was (85.7%), and as shown in [Table 6].

Table 2: Concentration of ATPO (IU/L) in serum of study groups

Groups	No.	Gender	ATPO Antibody * (IU/L)	
			Mean ± S.D	P.value
patient groups	22	Male	44.60 ± 5.80 a	**< 0.01**
	68	Female	59.77 ± 5.50 a	
Control group	14	Male	8.40 ± 5.09 b	
	36	Female	8.86 ± 6.81 b	

a, b: small letters refers to present significance differences (P <0.01) between groups at the vertical column.

* ATPO = anti-thyroid peroxidase.

Table 3: Concentration of H.pylori IgG among study group

Groups	No.	Gender	*H.pylori* IgG (IU/L)	P.value
			Mean ± S.D	
AITD *1	10	Male	92.10 ± 17.5 a	<0.01
	34	Female	92.57 ± 11.41 a	
Control	14	Male	48.89 ± 8.90 b	
	36	Female	53.25 ± 8.54 b	

a, b: small letters refers to present significance differences (P < 0.01) between groups at the vertical column.

*1= AITD: Autoimmune thyroid disease

Table 4: Concentration of cag-A IgG among study group

Groups	No.	Gender	Anti-cag-A IgG*1 (pg/ml)	P. value
			Mean ± S.D	
AITD*2	10	Male	25.45 ± 6.84 a	<0.01
	34	Female	26.25 ± 7.24 a	
control	14	Male	15.59 ± 4.69 b	
	36	Female	17.25 ± 5.07 b	

a, b: small letters refers to present significance differences (P < 0.01) between groups at the vertical column.

*1= Anti cytotoxin-associated gene A IgG

*2= AITD: Autoimmune thyroid disease

The proportion of female from 37 of AITD with positive *H.pylori* IgG were significantly higher (n = 29, 78.4%) than male patients which are revealed in [Table 6].

Discussion

In the current study, The results of the study recorded a significant increase in mean of TSH concentration ($P < 0.01$) in patients group particularly in Hashimoto's thyroiditis patients group, this finding was compatible with Abd Al Rahim MA, *et al.*,[20] who stated that a significant increase in the concentration of TSH documented in patients when compared with control participants. However, the level of ATPO in patients group was markedly higher than control group, also similar findings were found by Hamid ZA(2017).[21]

Our results of *H.pylori* IgG recorded in AITD patient group, so findings of present study, are in line with the majority of other studies represented that *H.pylori* IgG positivity was more prevalent among patients with autoimmune thyroid disease (AITD) than in other groups.[22,23]

However, the virulence factor of the bacteria has a great role in the pathogenesis of AITD progression as we found in the results of the our study that there was a significant increase ($P < 0.01$) in the level of anti-cag IgG in patients group as compared to control group, this result was in agreement with,[19,24] when they stated a significant association of virulent strain of *Helicobacter pylori* expressing cag A and autoimmune thyroid disease.

Previous reasearches explained that *H.pylori* CagA-positive strains have a highly similar sequence to thyroperoxidase, which is found in thyroid cells, so the infection with H. pylori may cause autoantibody damage to the intestinal mucosa, resulting in gastric disorders and antibody antigen cross-reactions causing thyroid tissue injury.[25]

Also, recent meta-analysis have demonstrated that the risk for AITDs increased in individuals with *H.pylori* infection when anti-CagA antibodies are present in their serum.[25]

The rate of infection in Hashimoto's thyroiditisgroup was greater than Graves' disease (G.D), this findings of our study, are compatible with other studies represented that the significant link between Helicobacter pylori and Hashimoto's thyroiditis suggests that this bacterium

Table 5: Distribution of H.pylori among patient groups and control group

Groups	Frequency & percentage	*Helicobacter pylori* IgG		Total
		Positive	Negative	
AITD	Freq.	37	7	44
	% Group	84%	16%	100%
	% *H.pylori*	39%	7%	46%
Non-AITD	Freq.	29	17	46
	% Group	63%	37%	100%
	% *H.pylori*	31%	18%	49%
Control	Freq.	28	22	50
	% Group	56%	44%	100%
	% *H.pylori*	30%	23%	53%
Total	Freq.	94	46	140
	% Group	67%	33%	100%
	% *H.pylori*	100%	100%	100%

Table 6: Distribution of *H. pylori* infection among H.T and G.D group

Group	Total	*Helicobacter pylori* IgG positive		
	No.	Total No. (%)	Male No. (%)	Female No. (%)
Hashimoto's thyroiditis (H.T)	27 (61.4%)	23 (85.2%)	6 (26%)	17 (74%)
Graves' disease (G.D)	17 (38.6%)	14 (82.4%)	2 (14.3%)	12 (85.7%)
Total	44 (100%)	37 (84%)	8 (21.6%)	29 (78.4%)

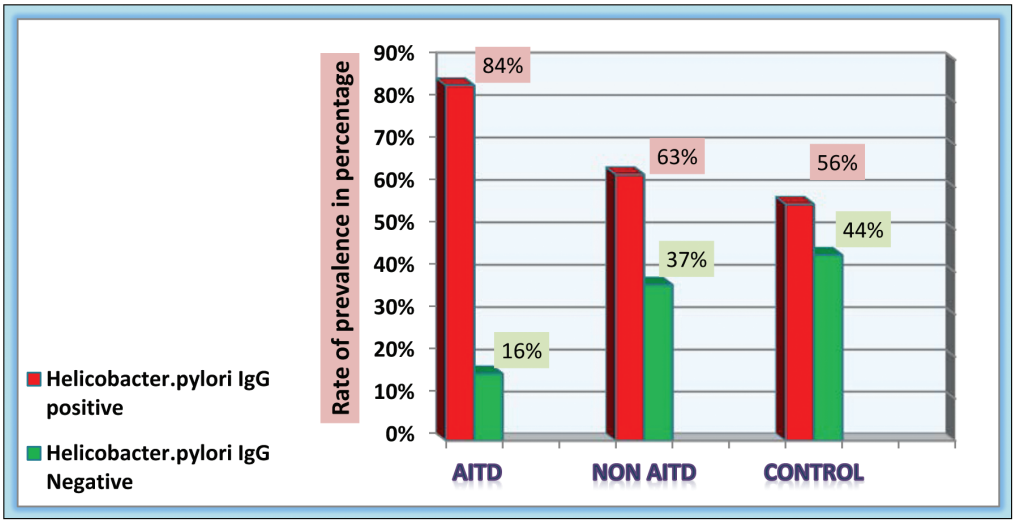

Figure 1: Prevalence of H.pylori infection among study groups

may play a role in the onset and/or maintenance of the autoimmune disorder.[26]

On the other side, some researches have provided a positive correlation between (G.D) and H. pylori infection.[27]

The results of the study also recorded the majority of patients with AITD were female, the results may contribute to the role of sex steroid hormones, such as estrogen, progesterone, and testosterone for promotion of the immune response to many of the sex-based disparities in the incidence of autoimmune disorders, and as such, they may play a part in this aspect.[28] Also We found that the risk of infection with *H.pylori* recorded higher in females than in males, this finding is in agreement with Abbas SK, *et al.*.[29]

Conclusion

Our findings indicated that thyroid gland can be a target for *H.pylori* by immune inflammatory responses which induced by impact of its virulence factor (cag A), so auto immune thyroid disease might be its consequence particularly in Hashimoto's thyroiditis.

Recommendations

The current study recommend that early diagnosis of *H. pylori* infection is important to avoid complication associated with the infection. And also, the Patients with *H.pylori* infection should be followed up by thyroid function tests to prevent such bacterial impact. Further researches must be designed by using other methods to investigate *H.pylori* infection. More studies are needed on the association between other autoimmune disorders and other bacterial infections.

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Conflicts of interest

There are no conflicts of interest.

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