

The Association between *Helicobacter pylori* and Graves' Disease

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Background and study aim:

Helicobacter pylori (*H. pylori*) infection is a very common health problem associated with both gastric and extra gastric manifestations. Its association with autoimmune thyroid diseases (ATD) including Graves' Disease (GD) was suspected and needed to be furtherly investigated.

Patients and Methods: This case - control study included 43 patients with GD and a control group of 47 healthy volunteers. Hormonal diagnosis of GD was achieved by decreased level of thyroid stimulating hormone (TSH) and elevated levels of tri-iodothyronine (FT3) and free thyroxine (FT4) and serological diagnosis was achieved by positive titers of auto-antibodies against thyroglobulin (TG

Abs), thyroid peroxidase (TPO Abs) and thyrotropin receptor (TR Abs). *H. pylori* infection was diagnosed by detecting *H. pylori* antigens in stool using an amplified enzyme immunoassay (amplified EIA). The antibodies against Cytotoxin-associated gene A (Cag-A) were assessed in serum samples using the enzyme-linked immunosorbent assay method (ELISA). The results were statistically analyzed using Fisher's test and the respective Odd's ratio (OR).

Results: No significant difference in prevalence of *H. pylori* infection was found between GD patients and the control group.

Conclusion: No association between *H. pylori* infection and Graves' disease could be detected.

INTRODUCTION

Helicobacter pylori (*H. pylori*) is a worldwide chronic infection [1,2]. It is a Gram - negative spiral pathogen that inhabits the gastric mucosa causing multiple gastric diseases such as chronic gastritis, peptic ulcers, and gastric malignancies [3,4]. It was also incriminated in both organ specific and non-organ specific autoimmune disease [5]. *H. pylori* was also involved in many extra gastric diseases such as nonalcoholic fatty liver diseases and metabolic syndrome [6,7]. Many available data links autoimmune thyroid diseases (ATD)- particularly Graves' disease (GD)- with *H. pylori* infection [8]. A strong association between thyroid auto- antibodies and immunoglobulin G (IgG) anti-*H. pylori* antibodies was reported. Radical treatment of *H. pylori* infection was found to be associated with a gradual drop in the levels of thyroid auto-antibodies, however these data are still controversial [9-11]. Moreover,

thyroid nodules were found to be associated with *H. pylori* infection in people with normal thyroid functions [12]. Cytotoxin-associated gene A (Cag A) strain accounts for most of *H. pylori* seropositive infection (89.2%) [13]. The mechanism by which *H. pylori* infection induces ATD was supposed to be a cross - reactivity between bacterial and thyroid antigens [14]. The ability of *H. pylori* infection to imitate the antigenic pattern of thyroid cell membrane has been suggested [15,16]. Multiple data - about similarities between amino acid sequence of *H. pylori* Cag-A and thyroid peroxidase (TPO) - have been published [17]. Typical markers of GD include the assessment of autoantibodies against thyroid peroxidase (TPO Abs), thyroglobulin (TG Abs) and thyrotropin receptor (TR Abs) [18].

This work aims at investigating the relationship between GD and *H. pylori* infection and whether there is any association in between.

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PATIENTS AND METHODS

This is a collaborate work between Tropical Medicine Department, Internal Medicine Department and Clinical Pathology Department, Faculty of Medicine Zagazig University. A one-year study was conducted in the period from January, 2017 to January, 2018.

A total number of 43 patients with GD (7 males and 36 females) and a control group of 47 healthy individual (27 females and 20 males) were enrolled in a case- control study over one year. The inclusion criteria included any patient with GD, while exclusion criteria included the presence of other diseases, history of antimicrobial drugs use for at least the preceding three months, presence of gastric diseases and dyspeptic symptoms (nausea, heartburn, and epigastric pain). Both groups were of the same body – mass index (BMI), socioeconomic status and inclusion criteria.

GD patients were selected based on their positive hormonal hyperthyroidism profile - including suppressed thyroid stimulating hormone (TSH), elevated free tri-iodothyronine (FT3), elevated free thyroxine (FT4) and positive titers of TPO Abs, TG Abs and TR Abs. They were measured by electrochemiluminescence immunoassay (ECLIA) on cobas immunoassay analyzers. Anti TG titer above 50 ng/ml and anti TPO above 200 IU/ml were considered positive. The control group individuals were confirmed to have normal TSH, FT3 and FT4, and to be free from autoantibodies against TPO, TG and TR. Also, thyroid ultrasound was done to assess the presence of goiter and to exclude thyroid nodules. Both groups were subjected to thorough history taking and clinical examination, then they underwent the following:

- *H. pylori* antigens were detected in fresh stool samples by amplified enzyme immunoassay (Amplified IDEIA *H. pylori* StAR, Oxoid, United Kingdom). Positive results were confirmed for the presence of *H. pylori* with an absorbance value >0.150 using a dual wavelength (450/620 to 650 nanometers).
- Anti - Cag-A IgG antibodies were detected in fresh serum samples by the enzyme-linked immunosorbent assay method (ELISA, Radim, Pomezia, Italy, sensitivity 93.7%, specificity 100%). An IgG level >15 units/mL was considered positive.

Statistical analysis:

The statistical analysis of results was carried out by an experienced epidemiologist. It was achieved using the SPSS version 19 considering P value <0.05 as statistically significant. The Chi-square or Fisher exact test were used to assess differences in proportions among categorical data. The independent impact of *H. pylori* on GD was assessed by multivariate analysis. On univariate analysis, variables with a P value of <0.2 were included in the multivariate analysis using Logistic regression for this purpose. Odds ratios (OR) with a confidence interval (CI) of 95% were reported. Median and interquartile range (IQR) were used to represent continuous variables.

RESULTS

A collaborate work was conducted over one year between Tropical Medicine Department and Internal Medicine Department, Faculty of Medicine Zagazig University. Forty-three GD patients were included in the study along with 47 healthy control individuals.

The study groups were classified into age and sex subgroups (range= 18-65 years). No statistically significant difference was found between both groups regarding age or sex (Table 1).

The mean, median and range of the body mass index showed no significant differences between the GD group and the control group (Table 2).

The prevalence of *H. pylori* +ve stool Ag was found to be more among GD patients' group (46.5%) than the control group (42.6%), but the difference was statistically insignificant. The prevalence of Cag A antibodies among the study groups was found to be more among *H. pylori* +ve stool Ag of the control group (30%) than the *H. pylori* +ve stool Ag of GD patients' group (20%), but the difference was statistically insignificant (Table 3).

H. pylori was more prevalent among the age subgroup of 40- 59 years of both GD patients and the control group but the difference was statistically insignificant (Table 4).

The prevalence of *H. pylori* was not associated with GD (OR 1.02, 95% CI 0.57–1.83, $P=0.95$), but the family thyroid malfunction was found to be a risk factor independently associated with GD (OR =3.93, 95% CI was 1.86- 618, and $P < 0.001$) (Table 5).

Table (1): Classification of patients and control groups according to age and sex.

Age \ Sex	GD group (n= 43)		Control group (n= 47)		P
	Female n= 36	Male n= 7	Female n= 27	Male n= 20	
18- 20 years	7 (19.4%)	0 (0 %)	7 (25.9%)	4 (20%)	0.159
>20- 39 years	10 (27.7%)	3 (42.8%)	9 (33.3%)	7 (35%)	
40- 59 years	8 (22.2%)	1 (14.2%)	6 (22.2%)	5 (25%)	
60- 65 years	11 (36.6%)	3 (42.8%)	5 (18.5%)	4 (20%)	

GD: graves' Disease.

Table (2): Comparison between the two study groups regarding the body- mass index (BMI).

Study groups \ BMI	GD N=43	Control N=47	P
Mean (SD)	25.5 ± (5.4)	25.2 ± (5.2)	0.801
Median (IQR)	24.6 (21.5- 28)	23.9 (21.2-28.9)	
Range	16.8- 44.4	16.4- 43.7	

IQR = interquartile ratio SD = standard deviation. GD: graves' Disease. BMI: body- mass index

Table (3): Comparison between the two study groups regarding the distribution of *H. pylori* stool Ag and serum Cag A-antibodies

H. pylori \ Study groups	Stool Ag +ve	Stool Ag -ve	Anti- Cag A +ve	Anti- Cag A -ve	+ve Anti- Cag A (among the total no. of studied groups)
GD n= 43	20 46.5%	23 53.5%	4/20 20 %	16/20 80 %	4/43 9.3 %
Control n= 47	20 42.6%	27 55.4%	6/20 30 %	14/20 70 %	6/47 12.7 %
P	0.63	Not calculated	0.352	Not calculated	0.41

Ag: antigen.

Cag A: cytotoxin-associated gene A.

GD: Graves' Disease.

Table (4): Comparison between different age subgroups regarding the prevalence of *H. pylori* infection

Age \ H pylori	GD group N=43	Control group N=47	P
18- 20 years	3/7 (42.8 %)	4/11 (36.3 %)	> 0.5
>20- 39 years	6/13 (46.1 %)	7/16 (43.7 %)	> 0.8
40- 59 years	5/9 (55.5 %)	6/11(54.5%)	> 1.0
60- 65 years	6/14 (42.8 %)	3/9 (33.3%)	>0.7

GD: Graves' Disease.

Table (5): Multivariate analysis of risk factors associated with GD.

Risk factors	OR (95% CI)	P
Univariate		
H. pylori	1.14 (0.66- 1.96)	0.63
Multivariate *		
- H pylori	1.02 (0.57- 1.83)	0.95
- Family history of thyroid diseases.	3.93 (1.86- 618)	< 0.001*

CI: confidence interval

OR: odd ratio

Graves' Disease

* adjusted for age

DISCUSSION

H. pylori is a Gram-negative motile organism that colonizes and infects the gastric mucosa causing multiple pathological conditions. Its virulence is mainly encountered with the Cag A antigen strains [19]. *H. pylori* - among other genetic or environmental factors - was involved in ATD [11,20,21]. ATD include GD, atrophic thyroiditis, Hashimoto's thyroiditis (HT), subacute lymphocytic thyroiditis (postpartum thyroiditis, PPT), painless thyroiditis (PT), or silent thyroiditis (ST) [22]. Accumulating information connected *H. pylori* infection with ATD, especially with GD [8], and thyroid nodular formation in euthyroid patients [12]. This study aimed at evaluating the association between *H. pylori* infection and GD.

This study showed that the prevalence of *H. pylori* was more among GD patients' group than the control group, but the difference was statistically insignificant. Interestingly, the prevalence of Cag A antibodies was even found to be more among the control group than the GD group, but the difference was statistically non-significant. These results agree with that of Tomasi et al. Franceschi et al and Novikova et al [23,27,28] who found no correlation between *H. pylori* prevalence and ATD in their studies directed on participants of different ages and different environmental conditions.

On the other hand, these results do not agree with that of some studies [8,11,13,15,24,29,37] who found marked correlation between *H. pylori* infection- especially the Cag A positive strains- and ATD especially GD. This can be explained by the different genetic or environmental factors of the different studies. In developing countries, the incidence of *H. Pylori* infection starts at earlier ages with eventual higher rates of prevalence than in developed countries that can mask the suggested association [8,24,25].

Moreover, certain HLA antigens were suspected to predispose for ATD following *H. Pylori* infection [26]. However, this association - in some studies - was not between GD and *H. Pylori* infection per se, but it was mainly between Cag A antibodies level and GD [25]. A cross reactivity was suggested between Cag A antibodies and TPO Abs explaining the over estimation of *H. pylori* infection among GD patients in these studies [14,17]. Some studies adopted the estimation of *H. pylori* antibodies as a clue for prevalence of *H. pylori* infection [11,29]. Their over estimation of *H. pylori* infection among GD patients can be attributed in some cases to a previous and not the current infection.

In this study, GD was found to be significantly associated with a family history of thyroid diseases. This finding coincides with that of other studies which mentioned that the incidence of GD clusters in families usually occurs separately or in association with HT [29-32]. This can be explained by sharing the same genetic or environmental factors by the family members.

There was no significant difference in *H. pylori* prevalence among the different age subgroups of both GD and control groups, coinciding with the study result of Haim et al who found no association between age and *H. pylori* prevalence [33]. However, these results do not agree with some authors [34-36] who mentioned that *H. pylori* infection is usually acquired during childhood and increases with age, with rare possibility to attract new infection in adulthood. Again, this controversy can be explained by the different genetic and environmental factors under which the different studies were conducted.

Conclusion: There is no association between *H. pylori* infection and Graves' Disease.

Ethical approval:

Informed consent was taken from each patient. The research protocol was duly approved by the ethical committee of Zagazig University Hospitals.

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Conflict of interest: None

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