Infection by Cag A Positive Strains of *Helicobacter pylori* is Associated with Autoimmune Thyroid Disease in Iranian Patients

Mahmood Soveid[1*], Kazem Hosseini Asl[2], Gholamhossein Ranjbar Omrani[3]

[1]Endocrinology and Metabolism Research Center, [2]Gastroenterology Unit, Department of Internal Medicine, [3]Endocrinology and Metabolism Research Center, Shiraz University of Medical Sciences. Shiraz, Iran

ABSTRACT

Background: Infectious agents have been suspected as a triggering factor for development of autoimmune thyroid disease (ATD). Some reports from Western countries have suggested association between *Helicobacter pylori* (HP) infection and ATD. **Objective:** To investigate the association of ATD with Cag A seropositivity in a population with high rate and early age of onset of HP infection. **Methods:** IgG anti HP and anti Cag A antibodies were measured in 88 patients with ATD and compared with results of 112 healthy individuals. **Results:** The rate of infection with HP was not significantly different in patient and control groups, but there was significant association between ATD and infection with Cag A strains ($p<0.005$). This association was significant for both hypothyroidism ($p<0.005$) and Graves' disease ($p<0.02$). Cag A antibody level correlated with titers of thyroid auto antibodies ($p<0.001$). **Conclusion:** In a population with high rate and early age of onset of HP infection, only infection with Cag A positive strains is associated with ATD, and this may be due to immune cross reactivity.

Keywords: Autoimmune Thyroid Disease, Cag A, Graves´ Disease, *Helicobacter pylori*

INTRODUCTION

Autoimmune thyroid disease (ATD) which includes chronic lymphocytic thyroiditis and Graves' disease is the most common autoimmune disorder (1). ATD arises due to interplay between environmental and genetic factors (2). Infectious agents such as *Yersinia enterocolitica* has long been suspected as a precipitating factor for development of ATD (3). It is hypothesized that antigenic similarity between these agents and thyroid can trigger autoimmune response through a mechanism of molecular mimicry (4).
Helicobacter pylori (HP) specifically colonizes the gastric epithelium and causes

Corresponding author: Dr. Mahmood Soveid, Endocrinology and Metabolism Research Center, Namazi Hospital, Shiraz, Iran, Tel: (+) 917 3158919, Fax: (+) 98 711 6474316, e-mail: msoveid@sums.ac.ir

chronic gastritis, peptic ulcer disease, and malignancy (5). The presence of the cytotoxin associated gene A (Cag A) antigen is a marker for virulence of the bacterium (6). Gastric infection by HP causes systemic immune response which has cross reactivity with thyroid (7). Some studies from Western countries have shown a positive correlation between HP infection and ATD (8-10), but others have denied such association (11,12), and this issue is still controversial (13). The aim of our study was to investigate the association between ATD and infection with HP in our population which has different epidemiologic characters with regard to HP infection (14).

MATERIALS AND METHODS

Patients. During a 2 year period between 2006 and 2008, 88 patients with ATD referring to endocrine clinics affiliated with Shiraz University of Medical Sciences were selected. Eighteen patients were male and 70 were female. The mean age of the patients was 35.9 ± 8.6 years (range: 18-63). They included 43 Graves' and 45 hypothyroid patients. Thyroid size was assessed by physical examination. Goiter was diagnosed according to WHO grading system and grades 1 and 2 were considered as Goiter (15). The diagnosis of Graves' disease was based on presence of elevated free T4 and T3 and suppressed TSH, and positive titer of thyroid receptor antibody. In cases with Graves' ophthalmopathy, eye involvement was evaluated according to Werner's classification (16) and patients with stage 2 or higher were considered to have ophthalmopathy. Autoimmune hypothyroidism was defined by elevated TSH and anti thyroid antibodies. Patients with history of thyroid surgery or radioactive iodine ablation were excluded. All patients were euthyroid at the time of study.

The control group consisted of 112 individuals (89 women, 23 men) with a mean age of 37.4 ± 6.1 years. They were age, gender, and socioeconomic class matched with the patient group. The controls had normal thyroid function tests and negative titers of anti thyroid antibodies.

The exclusion criteria for both patients and controls included: history of gastro duodenal disease, use of glucocorticoids and immune-suppressants during past one year, and drug treatment of HP. Written informed consent was obtained from patients and controls.This study was approved by the ethics committee of Shiraz University of Medical Sciences.

Antibody measurements: Anti thyroid peroxidase (anti TPO) and anti thyroglobuline (anti Tg) antibodies were measured by radio immunoassay (Zen Tech, Belgium). Anti Tg titer above 70 IU/ml and anti TPO above 50 IU/ml were considered positive. IgG anti HP was measured by Elisa (Monobind Inc. CA, USA). Values above 20 U/ml were regarded positive. Cag A-IgG was detected by Elisa (Dia. PRO. Diagnostic Bioprobes Srl. Milano, Italy). The test had sensitivity and specificity of > 98%. A concentration higher than 5arbU/ml was considered positive.

Statistical Analysis. Categorical data were analyzed by the Chi-square test with Yates' correction and an odds ratio (OR) with a 95% confidence interval was calculated. When the expected frequencies were small, Fisher test was used. Independent *t-test* was used to compare the means of two groups. Spearman's correlation coefficient was calculated to assess the relation between Cag A and thyroid auto antibodies. All data were analyzed by a computer program (SPSS, Chicago, IL, USA) and p-values less than 0.05 were considered significant.

RESULTS

HP antibody. Seventy five percent of the patients and 78% of the controls were positive for HP Ig G antibody and the difference was not significant. There was also no significant association between Graves' disease, hypothyroidism and HP infection.

Cag A antibody. Forty three (49%) of 88 patients with ATD were positive for Cag A antibody versus 28 (25%) of 112 controls ($p< 0.005$, OR: 2.87(CI: 1.58-5.21)).

Of 45 patients with hypothyroidism, 23(51%) had positive Cag A antibody which was significantly higher than controls ($p<0.005$, OR: 3.14(CI: 1.52-6.47)). This association was significant among patients with and without Goiter.

In patients with Graves' disease, 20(46%) of 43 patients were positive for Cag A antibody and the difference with controls was significant ($p< 0.02$, OR: 2.61 (CI: 1.25-5.45)).

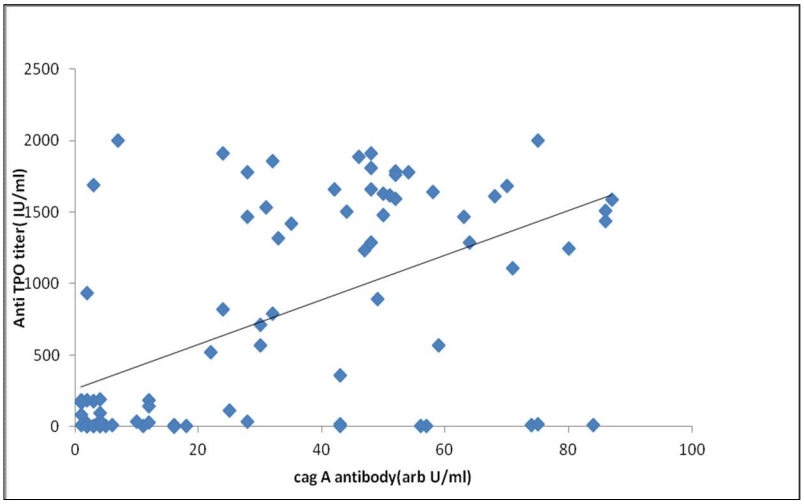

Figure 1. Correlation between Cag A and antibody and anti TPO titers; r=48, p<0.001.

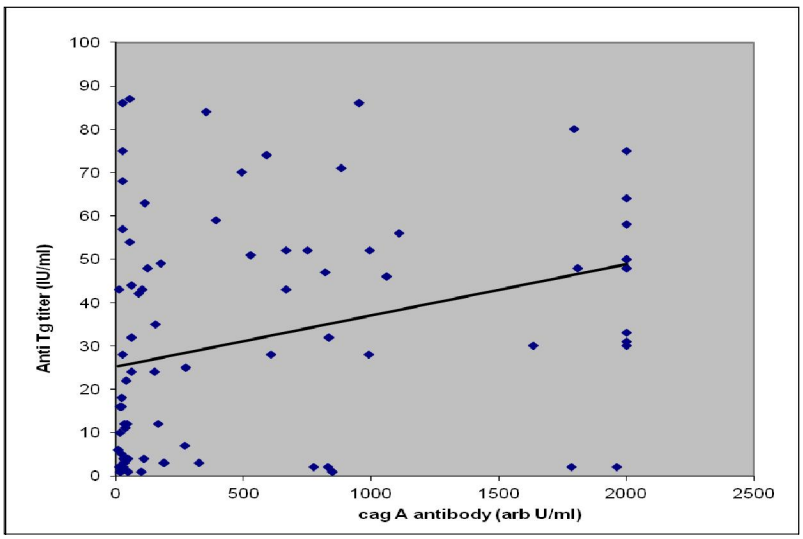

Figure 2. Correlation between Cag A antibody and anti Tg titers; r=0.38, p<0.001.

Among patients and controls with positive Cag A antibody, the mean value of antibody titer in patients was 44.9 ± 24 versus 28.2 ± 16.2 arb u/ml in controls (p=0.002).
There was significant correlation between Cag A antibody and anti TPO (r=0.48, p<0.001) and anti Tg (r=0.38, p<0.001) titers (Figures 1 and 2). The correlation between IgG anti HP antibody and thyroid auto antibodies was not significant.
There was no significant relation between Cag A antibody status and eye involvement in Graves' patients and presence of Goiter in hypothyroid patients.

DISCUSSION

Thyroid and the stomach have many embryologic and structural similarities and are involved simultaneously in some disease processes (17). The association between atrophic body gastritis and ATD is a well known example (18) and this has been attributed to the presence of shared epitopes between thyroid and gastric parietal cells (19). In some cases, HP can elicit autoimmune gastritis (20) and it has been suggested that through cross inactivity this can trigger thyroid autoimmunity (4,7). Antigenic similarity between HP and thyroid may also be involved in this process (7).Some studies from Western countries have reported positive association between HP infection and ATD (8,9). There has also been positive correlation between anti HP Ig G and anti thyroid antibody levels (8).
We found no significant relation between HP infection per se and ATD in our patients. There was also no correlation between IgG anti HP and anti thyroid antibodies in our study. This difference may be due to the different epidemiology of HP infection in developing countries. In Iran, HP infection occurs at an earlier age as compared with western countries (14) and this may have different consequences. The higher prevalence of HP infection in our population could also mask a significant association. The genetic background of the population can also be important, it has been shown that certain HLA antigens can predispose to ATD after HP infection (21). The lack of association between Graves' disease and HP infection per se may be due to early onset of HP infection in our population.
The significant finding of our study was the increased presence of Cag A antibody in patients with Graves' disease and hypothyroidism when compared with control group. This is compatible with some studies from Western countries (9,10). We also found significant correlation between Cag A antibody and anti thyroid antibody titers. Cag A positive HP strains carry some nucleotide sequences similar to thyroid peroxidase sequence (22). These data suggest a possible cross reaction between antibodies produced during HP infection and thyroid antigens leading to potential development of ATD and support the role of molecular mimicry in pathogenesis of thyroid autoimmunity (7). Significant reduction of anti thyroid antibodies after HP eradication (23) further supports a causal role for HP in development of thyroid autoimmunity.
We found no association between eye involvement in Graves' patients and Cag A positivity and this was in accordance with results of a similar study (10). In our hypothyroid patients there was significant association between infection with Cag A positive strains of HP and both goitrous and atrophic types of hypothyroidism. Franceschi et al. reported no association between HP infection and goitrous hypothyroidism (12) but De Luis et al. suggested an association between HP infection and atrophic hypothyroidism (8).

We suggest that in our population with a high rate and an early age of onset of HP infection, only the Cag A positive strains which can cause more severe and prolonged infection are associated with ATD.

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