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ORIGINAL ARTICLE

Association between *Helicobacter pylori* and Autoimmune Diseases Involving Type 1 Diabetes Mellitus and Autoimmune Thyroiditis

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ABSTRACT

Background: *Since the discovery of the harmful effects of Helicobacter pylori (H. pylori) and its initial discovery in the stomach, contemporary gastroenterology has made significant advancements. Epidemiological statistics indicate that autoimmune illnesses are more common when certain bacteria are infective or afterward. Studies have shown that H. pylori may be linked to different autoimmune disorders and that it may be a trigger for stomach autoimmunity. This study examines the current evidence supporting or refuting the theory that H. pylori may be a triggering factor for autoimmune disorders, such as type 1 diabetes mellitus and autoimmune thyroiditis.* ***Methodology:*** *Our study involved 180 participants divided into three equal groups including healthy control subjects, type 1 diabetes mellitus patients and autoimmune nondiabetic thyroid cases. All groups were subjected to blood sampling for measurement of Glycated hemoglobin A1c, thyroid stimulating hormone (TSH), free thyroxine (FT4), anti-H. pylori antibodies and cytokines multiplex test including IL-2, IL 18, and IFN gamma.* ***Result:*** *Serum IgG anti-H. pylori antibodies, IL 18, IFNγ levels showed higher levels while serum IL 2 level showed lower levels in autoimmune patients.* ***Conclusions:*** *Based on the aforementioned study, we conclude that H. pylori infection is one of the main environmental factors that contribute to multiple autoimmune illnesses.*

INTRODUCTION

Marshal and Warren were the pioneers in the isolation of *Helicobacter pylori* in 1983 from stomach biopsy[1]. Low socioeconomic status is positively correlated with the disease prevalence rate specially at childhood causing several health disorders including autoimmune gastritis, carcinoma and anemia[2].

For *H. pylori* to survive, it produces multiple virulence factors in order to tolerate the stomach's acidic environment such as the cytotoxin-associated gene A (CagA)[3]. Following its attachment to the gastric epithelial cells , *H. pylori* triggers the body's immune system, which is reflected in proinflammatory cytokines secretion by epithelial mucosal cells [4]. Among the serum cytokines are, interleukin-2 (IL-2), interleukin-18 (IL-18), tumour necrosis factor-α (TNF-α), and interferon-γ (IFN-γ), that are elevated both in the stomachs and serum of *H. pylori*-positive individuals [5].

Different microorganisms had been found to have a key role in immunity[4]. One of the main causes of autoimmune diseases is *H. pylori* infection[6].

Autoimmune disorders, such as Graves' disease (GD), Hashimoto's thyroiditis (HT) and others, that refocus on the thyroid as their target organ are known as Autoimmune Thyroid Disease (AITD). Its pathogenic mechanisms include the release of several cytokines, activation of the apoptotic gene ligand (Fas-L), local infiltration of B and T lymphocytes, inflammation and damage to cells and tissues [7]. Additionally, thyroid cells are harmed due to the combined cytotoxic effects of Tg Ab and thyroid peroxidase antibody (TPOAb). Numerous investigations indicate that *H. pylori* is associated closely with endocrine system disorders in addition to gastrointestinal disorders[8], where there is debate on whether *H. pylori* is among the variables producing AITDs.[8]

Regarding type 1 diabetes mellitus (T1DM), a number of factors (including infection, nutrition, etc.) are the primary causes. Viral infections, such as the cytomegalovirus was one of the clearest primary causes[9].

The question of whether *H. pylori* is correlated with diabetes remains unanswered [10].

A possibility of a clear correlation between *H. pylori* infection and autoimmune disorders remains up for debate. In order to assess and clarify the possible involvement of *H. pylori* in initiation and progression of autoimmune disorders, more researches on this subject is still required [11].

METHODOLOGY

The research ethics council at Cairo University authorized our case control study design (approval number: N-124-2023).

One hundred and eighty participants, ages 16 to 43, were enrolled in the Outpatient Diabetes and Rheumatology Clinic at Cairo University Hospitals in Egypt. Of these, 102 were female and 78 were male.

They were split up into three groups: Group 1: consisted of sixty healthy volunteers (26 men and 34 women) who were from the same area and matched in terms of age, sex, and socioeconomic status. These participants served as the controls for the study. Group 2: composed of sixty euthyroid T1DM patients (thirty-one males and twenty-nine females) who had been reported as having treated their diabetes for more than eight years and Group 3: consisted of 60 autoimmune thyroid cases (21 males and 39 females) that had previously received a clinical and laboratory diagnosis based on blood analysis indicating anti-TPO and/or anti-Tg positive.

Every participant signed an informed consent and completed a validated questionnaire to determine whether dyspeptic symptoms (epigastric discomfort, fullness, or sense of bloating) were present.

Criteria for exclusion:

No patients in groups 2 or 3 had issues associated with diabetes, and none of the patients in groups 2 or 3 had goiter or thyroid issues.

In the two months preceding testing, none of the patients in the three groups used prokinetics, proton pump inhibitors, antibiotics, or inflammatory drugs (which could exacerbate dyspeptic symptoms).

Sample processing: each blood sample was subjected to measurement of Glycated haemoglobin A1c, thyroid stimulating hormone (TSH), free thyroxine (FT4), anti-*H. pylori* antibodies and cytokines multiplex test including Il-2, IL 18, and IFN gamma.

A volume of 5 ml of heparinized blood samples were collected and separated into two tubes.

Hormonal and Glycated haemoglobin A1c assays were performed using the first tube of blood by DCA 2000 analyzer and an electro-chemiluminescent immunoassay utilizing the Roche Diagnostic Elecsys 2022 to quantify (HbA1c), thyroid stimulating hormone (TSH) and free thyroxine (FT4) respectively.

The other tube was centrifuged for 10 minutes at 1,500 g and 4°C for plasma separation and extraction to measure anti-*H. pylori* antibodies and cytokines multiplex test. Until they were analyzed, all samples were kept at -80 °C.

The sequential ELISA method, provided by Monobind Inc. (USA), was used to measure the IgG levels of serum anti-*H. pylori* antibodies. Patients in all 3 groups were deemed to have *H. pylori* when their anti-*H. pylori* IgG levels were found to be greater than 40u/ml. Plasma samples were centrifuged at 13,000 g for 5 min at room temperature to remove fibrin and debris before analysis in the bead-based assay.

Serum interleukins IL-2, IL 18, and IFN gamma were quantified simultaneously in plasma using an immune-enzymatic test provided by Luminex Corporate, Austin and BioRad, USA and employing a multiplex bead-based approach using monoclonal antibodies. This method allows multiple analyses to be measured simultaneously in a single experiment.

Both serum samples and standards were brought to room temperature, diluted with required buffer amount then vortexed. Antibody-coupled beads were made and diluted with assay buffer as directed by the manufacturer. We chose the taking after mAbs for coupling to distinctive fluorescent dots (MicroPlex Microspheres, Luminex Corp.): anti-human il-2 CC318 (IgG2b, MCA2110, BioRad); anti-human il-18 197-1 (IgG1); and anti-human IFN-γ CC330 (IgG1, MCA2112, BioRad).

Addition of equal amounts of solution to each well was done after a few tens of seconds of vortexing the diluted beads. After gently vortexing the prepared controls, blanks, standards, and samples, each well was filled with the same number of samples. The plate was placed in a microplate shaker, sealed and incubated for 20 minutes followed by rinsing of the dish three times after incubation.

An equal amount of streptavidin (SA-PE) solution was added to each well then the plate was sealed, covered with aluminum foil, and incubated on an oscillator.

Plate reading was done by centrifuging the previous complex to create a precipitate, wells content aspiration then a wash buffer was added, left to wash then reaspirated.

For reading the results, we mixed 100 µL of PBN into each well to resuspend the antibody beads. Then, we used a bead-based multiplex assay plate reader for reading the findings right away, and we placed the plate in an automated per user (BioPlex 200, BioRad).

Data were presented as mean fluorescence intensity (MFI), and the standard bent for each cytokine concentration was fitted using Wagner and More's (2009) computed 5p equation.

Serial weakening of the standard combination proteins and foundation values from measurements conducted virtually with test diluent were used to determine the measurement run.

The straight measurement run ranged from 20 to 130,000 pg/mL for IFN-γ, 95 to 620,000 pg/mL for IL-18, and 110 to 241,000 pg/mL for IL-2.

Statistical analysis

Data were encoded and entered using the statistical package for the Social Sciences (SPSS) version 28 (IBM Corp., Armonk, NY, USA). Data summerization was done using mean, standard deviation, median, minimum and maximum in quantitative data and using frequency (count) and relative frequency (percentage) for categorical data. Comparisons between quantitative variables were done using the non-parametric Kruskal-Wallis and Mann-Whitney tests (Chan, 2003a). For

comparing categorical data, Chi square ($\chi 2$) test was performed. Exact test was used instead when the expected frequency is less than 5 (Chan, 2003b). Correlations between quantitative variables were done using Spearman correlation coefficient (Chan, 2003c). ROC curve was constructed with area under curve analysis performed to detect best cutoff value of significant parameters for detection of autoimmune thyroiditis. P-values less than 0.05 were considered as statistically significant.

RESULTS

To assess the possibility that *Helicobacter pylori* might be an etiologic agent of autoimmune diseases, titers of anti-*H. pylori* IgG in sera of patients in two groups were compared with those of control group by sequential ELISA method (table 1)

Table 1: Comparison between the control Group (1), type 1 DM group (2) and autoimmune thyroid disease group (3) as regard the gender, free-thyroxine (FT4), thyroid stimulating hormone level (TSH), Glycated Haemoglobin A1c level (HbA1c), Serum anti *H. pylori* antibodies, serum IL-2, serum IL-18 and serum IFNγ level

		Group 1 control		Group 2 DM 1		Group 3 autoimmune thryroiditis		P value
		Count	%	Count	%	Count	%	
Gender	**F**	34	56.7%	29	48.3%	39	65.0%	0.183
	M	26	43.3%	31	51.7%	21	35.0%	
FT4	**normal**	60	100.0%	56	93.3%	0	0.0%	0.119
	high	0	0.0%	4	6.7%	0	0.0%	
TSH	**normal**	60	100.0%	56	93.3%	0	0.0%	0.119
	high	0	0.0%	4	6.7%	0	0.0%	
Glycated hemoglobin A1c (HbA1c)	**normal**	58	96.7%	9	15.0%	42	70.0%	< 0.001
	high	2	3.3%	51	85.0%	18	30.0%	
Serum anti-H. pylori antibodies IgG	**normal**	50	83.3%	15	25%	14	23.3%	< 0.001
	high	10	16.7%	45	75%	46	76.7%	
Serum Il-2	**normal**	57	95.0%	43	71.7%	42	70.0%	0.001
	low	3	5.0%	17	28.3%	18	30.0%	
Serum il-18	**normal**	50	83.3%	11	18.3%	16	26.7%	< 0.001
	high	10	16.7%	49	81.7%	44	73.3%	
Serum IFNγ	**normal**	51	85.0%	25	41.7%	19	31.7%	< 0.001
	high	9	15.0%	35	58.3%	41	68.3%	

Titers of Anti-*H. pylori* antibodies in autoimmune disease patients namely T1DM and patients with AITD and from age matched healthy controls were determined

OF 60 control 10 (16.7%) showed high serum IgG anti-*H. pylori* antibodies level, In the disease groups the level was high in 45 (75%) out of 60 patients with type 1 DM and in 46 (76.7%) out of 60 patients with AITDs and these results were statistically significant

As regards cytokines level serum IL 2 level, it was low in 17 (28.3%), 18 (28.3%) patients with type 1DM and patients with AITD respectively compared to 3 (5%) in the control group and these results were statistically significant.

Serum IL 18 level was high in 49 (81.7%), 44 (73.3%) patients with type 1DM and AITD respectively compared to 10 (16.7%) in the control group and these results were statistically significant.

Serum IFNγ level was high in 35 (58.3%), 41 (68.3%) patients with type 1DM and AITD respectively compared to 9 (15%) in the control group and these results were statistically significant.

Upon comparing The 3 groups regarding *H pylori* antibodies level, IL-2 level, IL-18 serum levels and IFNγ level using Kruskal-Wallis test there was a mean statistical difference between group 1 and 2 also between group 1 and 3 while no statistical significance was found on comparing group 2 and 3 (table 2)

Table 2: Independent sample Kruskal-Wallis test for distribution of mean level of Serum anti *H.pylori* antibodies, IL 2, IL 18 and IFN γ between the 3 groups

		P value
Serum anti *H.pylori* antibodies between the 3 groups	Group 1 control-Group 2 DM 1	**< 0.001**
	Group 1 control-Group 3 autoimmune thryroiditis	**< 0.001**
	Group 2 DM 1-Group 3 autoimmune thryroiditis	**1.000**
Serum IL 2 between the 3 groups	Group 3 autoimmune thryroiditis-Group 2 DM 1	1.000
	Group 3 autoimmune thryroiditis-Group 1 control	0.002
	Group 2 DM 1-Group 1 control	0.007
Serum IL-18 between 3 groups	Group 1 control-Group 3 autoimmune thryroiditis	< 0.001
	Group 1 control-Group 2 DM 1	< 0.001
	Group 3 autoimmune thryroiditis-Group 2 DM 1	1.000
Serum IFN γ between 3 groups	Group 1 control-Group 3 autoimmune thryroiditis	< 0.001
	Group 1 control-Group 2 DM 1	< 0.001
	Group 3 autoimmune thryroiditis-Group 2 DM 1	0.589

When comparing serum Il2, serum Il 18 and serum IFN γ with the level of serum anti-*H.pylori* antibodies Ig in patients in group 2 patients with type 1 DM, P value was statistically significant (table 3)

Table 3: Correlation between Serum anti-*H. pylori* antibodies Ig and other parameters in DM1

	Group 2 DM 1		
	Serum anti-*H. pylori* antibodies Ig G (u/ml)		
	Correlation Coefficient	**P value**	**N**
FT4 (ug/dl)	0.007	0.960	60
TSH (mIU/L)	0.183	0.161	60
Glycated hemoglobin A1c (HbA1c)	0.038	0.772	60
Serum Il-2 (pg/ml)	-0.501-	< 0.001	60
Serum il-18	0.355	0.005	60
Serum IFN γ (pg)	0.271	0.036	60

P value was statistically significant when comparing Glycated hemoglobin A1c**,** serum IL2, and serum IL 18 with the serum level of anti-*H. pylori* antibodies Ig in patients with AITD (table 4)

Table 4: Correlation between Serum anti-*H. pylori* antibodies Ig and other parameters in autoimmune thyroid disease

	Group 3 autoimmune thryroiditis		
	Serum anti-*H. pylori* antibodies Ig G (u/ml)		
	Correlation Coefficient	**P value**	**N**
Glycated hemoglobin A1c (HbA1c)	0.358	0.005	60
Serum Il-2 (pg/ml)	-0.599-	< 0.001	60
Serum il-18	0.527	< 0.001	60
Serum IFN γ (pg)	0.325	0.011	60

On determining ROC curve for prediction of autoimmune disease using serum anti-*H. pylori* antibodies IgG, IL -2, IL-18, IFN γ cutoff value that were statistically significant were less than 42, 2.85 , 114.5 and 73.3 respectively (tab 5, Fig1 &2)

Table 5: ROC curve for prediction of autoimmune disease using Serum anti-*H. pylori* antibodies IgG, il-18, IFN γ and il-2

	Area Under the Curve	P value	95% Confidence Interval		Cut off	Sensitivity %	Specificity %
			Lower Bound	Upper Bound			
Serum anti-*H. pylori* antibodies Ig G (u/ml)	0.832	< 0.001	0.756	0.907	> 42	78.3	85
Serum IL-2 (pg/ml)	0.686	< 0.001	0.592	0.781	< 2.85	75	55
Serum IL-18	0.842	< 0.001	0.759	0.925	> 114.5	86.7	81.7
Serum IFN γ (pg)	0.840	< 0.001	0.767	0.913	> 5.05	73.3	85

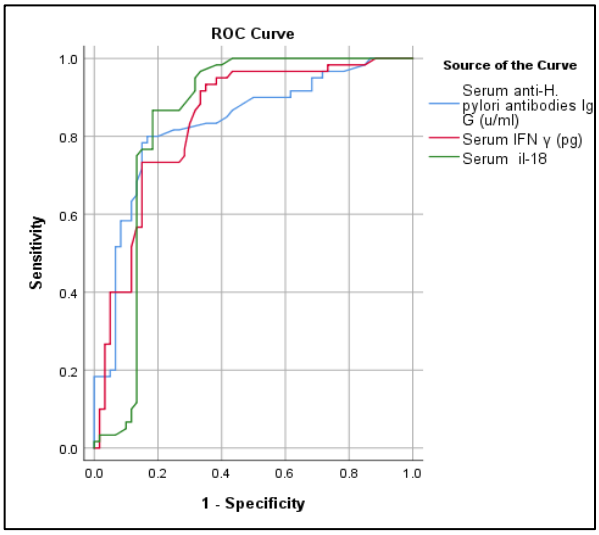

Fig.1: ROC curve for prediction of autoimmune disease using serum anti-H. pylori antibodies IgG, il-18, IFN γ

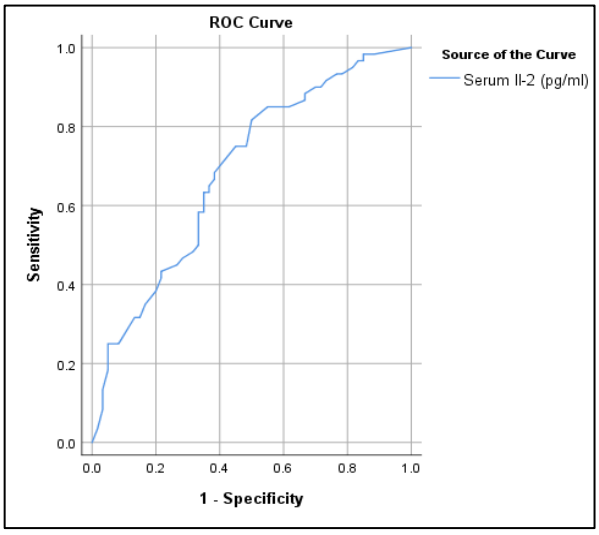

Fig. 2: ROC curve for prediction of autoimmune disease using serum Il-2

DISCUSSION

According to epidemiological studies, autoimmune illnesses appear to be higher during or after infection with certain bacteria. It has been shown in studies that *H. pylori* was associated with cytokine serum level alterations as well as other innate and acquired autoimmune illnesses, and was a trigger for stomach autoimmunity. This study investigates the body of evidence supporting the hypothesis that *H. pylori* is an etiologic agent of autoimmune diseases.

Additionally, we looked for changes in specific cytokines serum level that could indicate the onset of an autoimmune illness in those with seropositive *H. pylori* antibodies.

For the past few years, there has been a debate regarding the correlation between *H. pylori* infection and diabetes. Our findings were corroborated by several investigations.

Some studies found *H. pylori* infection to be positively associated with diabetes [12- 15].

Our study revealed that there is an association between *H. pylori* antibodies and T1DM where 45 patients (75%) in our study were found positive with *H. pylori* antibodies

Our results are supported by a study that was conducted in Sudan on children with diabetes aged 11 to 18 years. Of the 41 newly diagnosed diabetic children (44.4%) who complained of symptoms, 30 (53.6%) were seropositive for *H. pylori*, compared to 34 (37.7%) among the healthy children, of whom 24 (40.7%) tested positive for *H. pylori*. The study also included formally diagnosed patients, 32/46 (57%) of them were seropositive for *H. pylori* [16].

According to another study[17], Anti- *H. pylori* IgG was positive in 49/88 (55.6%) of diabetics and 13/42 (30.9%) of controls. These findings corroborated our findings.

A study assessing the *H. pylori* infection impact on insulin requirement in diabetic individuals reported that infected children need more insulin (1.2 versus 0.9 IU/Kg/d) and their glycosylated haemoglobin. Given our findings that there is a strong correlation between *H. pylori* and diabetes patients, the amount was greater

(14.9 vs. 11.8) than the level obtained in uninfected people [18].

Zekry et al.[19], similarly found a positive relationship between HbA1 c and anti-*H pylori* IgG antibody levels. *H. pylori* -infected diabetic children required treatment with higher insulin dosages and recorded higher HbA1 c .

Due to the increased production of pro-inflammatory cytokines generated by the *H. pylori* stomach infection itself, *H. pylori* infected patients may have poor glycemic control. Conversely, changes in glucose metabolism could encourage the colonization of *H. pylori* [20].

The increased incidence of *H. pylori* infection in patients with diabetes is typically attributed to decreased stomach motility and peristaltic activity, which may encourage *H. pylori* colonization and different chemical gastric mucosal alteration due to non-enzymatic glycosylation of mucins or elevated sialic acid, which may function as a cell surface receptor for *H. pylori* by encouraging the bacteria's adhesion to gastric mucosa cells; additionally, diabetic patients have been shown to have impaired non-specific immunity [21].

Additional research[22,23] showed that there is no association between *H. pylori* infection and diabetes.

According to a study[24], *H. pylori* infection frequency in T1DM patients was 17/63 (27%) and in the control group was 25/105 (23.8%), which was not consistent with our findings.

In a study on 116 patients and 50 healthy control subjects, no statistically significant difference was present between the diabetic patients and controls, with *H. pylori* infection being similar in IDDM patients (37%) compared to the healthy group (34%).[25]

Noteworthy a correlation between *H. pylori* infection and autoimmune thyroid illness of the 6 autoimmune thyroid disorders patients was reported in our study, 46 (76.7%) had elevated serum IgG anti-*H. pylori* antibodies and these results were statistically significant.

Similar to our results, the results of a research on adult female patients reported an association between autoimmune thyroid illness and a persistent *H. pylori* infection. [26]

On line with our research, a meta-analysis comprising fifteen studies demonstrated that AITD patients exhibited a greater frequency of *H. pylori* infection compared to those without the condition, and it was found that the removal of the infection can decrease the corresponding autoantibodies. [27]

In a study conducted on 47 women with autoimmune thyroiditis, seropositivity was found in 46.5 percent of them, compared to 4% of the 48-control group who tested positive. [28]

Also supporting our results, a meta analysis that included seven studies with a total of 862 participants, concluded that the association was significant for Graves' disease. Additionally, it was mentioned that the chance of developing autoimmune thyroid illness was elevated by 2,24 times with seropositivity for *H. pylori* antibodies. [29]

Another investigation that corroborated our findings showed seven (8.0%) out of thirty-two patients tested positive for *H. pylori* infection, whereas sixteen controls (24.3%) tested positive. [30]

In keeping with our research, a second study was conducted with 136 controls and 76 cases of Hashimoto thyroiditis, 44 cases of specific thyroiditis and 39 cases of Graves' disease. Infection was detected in 64.4% of cases of Hashimoto thyroiditis and 26.6% of cases of Graves' illness, while 34.0% of cases of specific thyroiditis and 29.4% of controls were seropositive.[31]

Proinflammatory cytokine release is elevated in CagA-positive AITD patients, which supports the theory that pathogenic *H. pylori* strains may either cause the autoimmune response to flare up or act as an initiator by enhancing the release of IL-6, IL-18 and other proinflammatory cytokines associated with increased levels of CagA antibodies and autoantibodies.[32,33]

For linking between *H. pylori* infection and autoimmune illnesses, a trial involving associated cytokine assessment was conducted in our study.

Our research on IL-2 showed that serum IL-2 levels were low in 17 (28.3%) of patients with T1DM and 18 (28.3%) of patients with AITD, respectively, compared to 3 (5%) in the control subjects. We also found that autoimmune illness can be predicted at threshold less than 2.85 pg/ml.

According to Sundrud et al. [34], there is an evidence to support the explanation that the *H. pylori* vacuolating toxin (VacA) suppresses T cell activation by the down regulation nuclear factor of activated T cells (NFAT), hence preventing T cell synthesis of IL-2. According to Togawa et al. [35], there is a strong association between *H. pylori* infection and IL-2 gene polymorphism and a high risk of inducing gastric atrophy.

Serum IL-18 levels above 114.5 pg/ml can indicate the presence of autoimmune illness when determining the ROC curve for autoimmune disease prediction.

Similarly, it was discovered that *H. pylori*-induced IL-18 plays a significant role in gastric injury and was found to be positively correlated with the degree of gastric inflammation, and IL-18 levels were found to be significantly elevated in *H. pylori*-infected epithelial cells and monocytes. [36]

A research on the linkage between IL-18 and diabetes metabolic control has demonstrated a favorable correlation between IL-18 and IL-18BP with HbA1c levels in T1D patients. [37]

CONCLUSION

Based on our study, we can conclude that *H. pylori* infection is one of the major environmental factors that contribute to several autoimmune illnesses.

Finally, utilizing serum anti-*H. pylori* antibodies IgG, IL-2, IL-18, IFN γ with the indicated cutoff values > 42 u/ml, < 2.85, > 114.5, and > 5.05 pg/ml respectively can aid in the diagnosis and screening of autoimmune disorders, it is positively connected with T1DM and AITD.

Future research must determine and validate the effect of *H. pylori* on the likelihood and course of different autoimmune disorders.

Declarations:

Consent for publication: Not applicable

Availability of data and material: Data are available upon request

Competing interests: The author(s) declare no potential conflicts of interest with respect to the research, authorship and/or publication of this article none.

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