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Incidence of *Helicobacter pylori* Infection among Hashimoto's Thyroiditis Patients in Amara City, Iraq

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Abstract

The association of different infections with the subsequent occurrence of autoimmunity, just like autoimmune thyroiditis, has recently been increased globally. This study aimed to determine the role of *Helicobacter pylori* (HP) infection in Hashimoto's disease. The research groups involved 50 patients with Hashimoto's disease and 50 healthy subjects. All of them were subjected to the estimation of concentrations of free triiodothyronine (FT3), free thyroxine (FT4), TSH, anti-thyroid peroxidase (anti-TPO), and anti-thyroglobulin (anti-Tg). In addition, for the diagnosis of HP, IgG, as well as anti-CagA antibodies in the serum, were detected. An independent t-test was used to test the significance of the means. Also, an odd ratio was used to clarify the association between *H. pylori* infection and HT disease. Statistical significance was detected when *P-value* is equal to or less than 0.05. The results indicated that 94% of Hashimoto's patients and 34% of the healthy subjects were seropositive for HP IgG. Also, (74%) of the HT patients were seropositive for HP IgG/anti-CagA. These results were significant at the level of 0.01 ($p< 0.001$). Hashimoto's patients with HP/CagA positive tests have significantly elevated concentrations of anti-TPO (480.69±311.29), anti-Tg (336.00±175.95), and TSH (20.43±18.98) compared with patients tested negatively to HP/CagA antibodies (358.60±281.55, 258.36±170.09 and 9.02±5.94 respectively). In conclusion, there is a relationship between *H. pylori* infection and the development of Hashimoto's thyroiditis in Iraqi patients. *H. pylori* infection, especially CagA expressing strains, could be a risk factor for the development of autoimmune hypothyroidism and to a lesser extent its progression by increasing the concentration of thyroid antibodies and TSH, which in turn leads to decreased levels of the thyroid hormones and worsening of the disease, requesting antibiotic therapy to eradicate the bacterial infection.

Keywords: Hashimoto's thyroiditis, *H. pylori infection,* correlation, anti-TPO, anti-Tg, CagA.

1. Introduction:

Autoimmune thyroid diseases (AITDs) are many distinct clinical disorders, of which Hashimoto's hypothyroidism (HT) and Graves' hyperthyroidism are the most prominent (Caturegli *et al*., 2014). They reflect examples of autoimmune organic-specific diseases which are restricted to the thyroid gland. HT is highly abundant in a female with an incidence ratio of about 8:1(Casto *et al*.,2021). However, according to the positive results of laboratory tests in women for the occurrence of autoantibodies for thyroid, about 10% of the population are suffering from HT (Machała *et al*.,2019). In the pathogenesis, the thyroid antigens may be presented by dendritic cells as foreign antigens to the T-cells leading to its proliferation and differentiation into thyroid-specific T-cells (Th1, Th2, and CD+8) producing different cytokines like IL-12, IL-17, and IFN-α which in turn mediate thyroid infiltration and cytotoxicity (Ramos-Leví, and Marazuela, 2016; Machała *et al*.,2019). Although the exact cause of AITD is unknown, they are genetically expressed and require an environmental trigger (Ragusa *et al*.,2019). Infection with *Helicobacter pylori* (HP), a gram-negative, motile, microaerophilic bacteria, is a potential environmental factor, causing persistent inflammation and immunological response in vulnerable individuals (Choi *et al*.,2017).

Studies have revealed that levels of anti-*H. pylori* cytotoxin-associated gene A product (anti-CagA) are significantly higher in HT patients than in healthy controls (Figura *et al*.,1999). CagA expressing strains of *H. pylori* have higher inflammatory activity, and they raise inflammatory cytokine levels in the stomach and throughout the body in an infected person, which may be associated with the extra-intestinal consequences of the host tissues and the subsequent development of autoimmunity (Figura *et al*., 2020). However, researchers are unable to clarify the *H. pylori-related* consequences that can cause thyroid autoimmunity. Also, the specific processes by which exposure to a microorganism induces more than one manifestation of autoimmunity (Hamid,2017; Hou *et al*., 2017). Delitala *et al* have suggested that *H. pylori* may expose the host's sequestered epitopes to the immune system leading to the development of autoimmune reaction (Delitala *et al*.,2016). In this context, the current study aimed to investigate the correlation between *H. pylori* infection and Hashimoto's thyroiditis and to evaluate if there is an effect of *H.*

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pylori on the serum levels of thyroid hormones and TSH in the patients.

2. Materials and Methods

The present study involved 100 persons (males and females) aging between (9-50 years) during the period from Dec. 2019 to Dec. 2020. Study participants were classified into two groups. The 1st group (50 patients) attended the specified center of diabetes and endocrine glands diseases in Amara city with symptoms suspected to have Hashimoto thyroiditis which was confirmed using serological tests for the detection of anti-TPO and anti-Tg. The second group (50 persons) were healthy persons of comparable age and sex and considered a control group. They were all subjected to serologic tests for the diagnosis of autoimmune thyroiditis. All of the study participants have read and signed the patient consent form, and the study has been approved by the Committee of Scientific Research Ethics / Amara Medical Institute.

3. Sample collection

Ten ml of venous blood was collected from all the study participants then centrifuged at 5000 rpm /min for 5 mins and the obtained sera were used for the serological methods for the estimation of thyroid antibodies (Anti-TPO and Anti-Tg) as well as thyroid hormones (FT3, FT4) and TSH.

4. Estimation of thyroid hormones levels in the serum

Serum levels of the thyroid hormones free triiodothyronine (fT3), free thyroxine (fT4), and thyroid-stimulating hormone (TSH) were determined on the same day of blood collection by using the electrochemiluminescence immunoassay method (Cobas, comp. Penzberg, Germany). The results were expressed in IU/mL as per the manufacturer's instruction.

5. Detection of thyroid auto-antibodies in the serum

Serum concentrations of anti-Tg and anti-TPO were evaluated using a chemiluminescent immunoassay (Mindray, China). As per the instructions of the manufacturer, the results have been recorded in IU/mL.

6. Detection of H. pylori infection

H. pylori infection in HT patients and control group has been diagnosed by using two ELISA kits (Hp-IgG, Monobind Inc. USA and anti-CagA, Sunlong Biotech. China). Biotech ELISA reader and washer (Biotech, USA) was used, and the procedure was applied; results were achieved as per the manufacturer's instructions.

7. Statistical analysis

Results of the current study were analyzed using SPSS software package ver.23 (performed by IBM Co. USA). An independent t-test was used to test the significance of the means. Also, an odd ratio was used to clarify the association between *H. pylori* infection and HT disease. Statistical significance was considered when *the P-value* is equal to or less than 0.05.

8. Results

The current study is a case-control study that involved (50) HT patients and (50) healthy controls. All of the study participants were subjected to the estimation of serum FT3, FT4, TSH, anti-TPO, and anti-Tg. The results found a significant increase in serum TSH concentrations (P <0.001) in HT patients compared to healthy subjects as shown in table (1). Serum FT4 and FT3 concentrations were also elevated in HT patients compared to the control group which is not statistically significant.

Table 1: Serum concentration of thyroid hormones in HT patients and healthy controls.

Hormones	HT group Mean ± SD	Control group Mean ± SD	*P* value
FT3 (pmol/L)	4.76±1.50	7.84±4.39	0.031*
FT4 (pmol/L)	12.07±4.27	15.57±3.58	0.056*
TSH (µIU/ml)	18.53±17.60	2.45±1.50	<0.001**

**Results are significant at 0.01 level. *Results are significant at 0.05 level. SD: standard deviation

The levels of both of anti – TPO antibodies, as well as anti - Tg antibodies, are significantly increased (*P-value* < 0.001) in HT patients against the healthy group, table (2).

Table 2: Serum concentration of thyroid auto-antibodies in the study groups.

Thyroid antibodies	HT patients Mean ± SD	Control Mean ± SD	*P* value	Reference value
Anti-TPO (IU/L)	424.79 ± 381.90	1.92 ±0.08	<0.001**	(≤ 9IU/ml)
Anti-Tg (IU/L)	56.76 ± 15.41	3.59 ±5.07	<0.001**	(≤ 4IU/ml)

**Results are significant at 0.01% level. SD: standard deviation

The incidence of *H. pylori* antibodies (IgG, CagA) among HT patients and the healthy control group is described in table (3). The HT patients with positive *H. pylori* IgG were significantly higher (47 of 50, 94%) compared to the healthy subjects (17 of 50, 34%). These results were highly significant (OR: 7.01, *P-value* < 0.001). Similarly, samples with positive results for both HP-IgG and anti-CagA represented (37%) of the HT patients and overall study groups, as all of the healthy subjects were seronegative for anti-CagA antibody. On the other hand, only (17, 34%) of the HT group showed positive *H. pylori* IgG results and negative anti-CagA against (20%) in the control group. These results were insignificant (OR: 1.83, P: 0.18).

Table 3: Incidence of HP-IgG and anti-CagA antibodies among HT patients and control group

H. pylori IgG / CagA status	HT n=50	Control n=50	*P* value	OR	95% CI
	No. (%)	No. (%)			
HP+	47(94%)	17(34%)	< 0.001**	7.01	3.00 to 16.39
HP-	3(6%)	33(%66)	< 0.001**	0.03	0.008 to 0.121
HP+/CagA+	37(74%)	0 (0%)	< 0.001**	280.55	16.16 to 4870.44
HP+ / CagA -	17(34%)	10(20%)	0.18	1.83	0.83 to 5.10

**Results are significant at 0.01% level. OR: odd ratio. 95% CI: confidence intervals.

The results of table (4) showed that TSH levels were non-significantly elevated in HT patients who had *H. pylori* IgG (HP+) in their sera in comparison with those with (HP-) sera. As a result, levels of the thyroid hormones (FT3 and FT4) were insignificantly lower in HT patients who tested positive for HP IgG compared to those who tested negative. In HT patients who tested positive for both *H. pylori* antibodies (HP+/CagA+) the levels of TSH were significantly higher (*P* value=0.008) compared to those with HP+/CagA- tests. As a consequence, there was an insignificant decrease in the levels of thyroid hormones (FT3 and FT4) in these patients compared to those with (HP+/CagA+) results, table (4).

Table 4: Serum levels of FT3, FT4 and TSH in HT patients according to the type of *H. pylori* antibodies.

Thyroid hormones (Mean± SD)	*H. pylori* antibodies					
	HP +	HP -	P value	HP+/CagA+	HP+/CagA-	P value
FT3	4.58 ± 1.44	4.76 ± 1.49	0.94	5.43 ± 1.56	4.58 ± 1.44	0.805
FT4	11.96 ± 4.42	12.00 ± 4.31	0.943	11.14 ± 4.08	11.96 ± 4.42	0.801
TSH	20.34 ± 15.98	17.93 ± 11.63	0.457	20.43 ± 18.98	9.02 ± 5.94	0.008**

*Results are significant at 0.05 level. SD: standard deviation.

In the same context, there is a considerable elevation (*P-value* =0.05) in the concentrations of anti – TPO among HT patients who tested positive for both *H. pylori* antibodies (IgG and anti-CagA) when compared with those who tested negative, table (5). Serum levels of anti – Tg on the other hand, have also been elevated in the same groups but this elevation was not statistically significant.

Table 5: Serum levels of anti-TPO and anti-Tg in HT patients according to the type of *H. pylori* antibodies.

Thyroid antibodies (IU/ml)	*H. pylori* antibodies					
	HP +	HP -	P value	HP+/CagA+	HP+/CagA-	*P* value
Anti-TPO (Mean ± SD)	443.62 ± 386.72	106.65 ± 20.40	0.05*	480.69 ± 311.29	358.60 ± 281.55	0.05*
Anti-Tg (Mean ± SD)	336.00 ± 175.95	330.00 ± 190.95	0.943	336.00 ± 175.95	258.36 ± 170.09	0.721

*Results are significant at 0.05 level. SD: standard deviation.

9. Discussion

Our results found that serum levels of TSH, thyroid autoantibodies were significantly increased in HT patients when compared with normal control. Also, thyroid hormones (FT3 and FT4) were elevated in HT patients, but this elevation was not significant. These results could be considered as an indication for the development of subclinical hypothyroidism which is characterized by elevated TSH levels and normal FT3 and FT4 levels (Machała *et al*.,2019).

For the diagnosis of HT, the current study was dependent on the estimation of serum concentrations of anti-TPO and anti-Tg antibodies; the results indicated, as shown in table (2), that the concentrations of these antibodies were significantly higher (*P*<0.001) in patients compared to the healthy controls. Elevated concentrations of the thyroid autoantibodies and TSH as well as normal levels of thyroid hormones (FT3 and FT4) indicated the development of subclinical HT disorder. Anti-TPO and anti-Tg antibodies are widely available and commonly used in clinical diagnostic laboratories for HT disease (Wang *et al*., 2018). These antibodies are the major anti-thyroid antibodies in Hashimoto's disorder, and growth in anti-TPO antibodies has been linked to clinical symptoms of illness progression in the future (Acar *et al*., 2013). Furthermore, Siriwardhane *et al*. (2019) concluded that serum levels of anti-Tg and anti-TPO can be used as markers for early prediction of the development of thyroid autoimmunity; they also recommend adding these tests to the same list of thyroid function tests which include FT3, FT4, and TSH. On the other hand, (10%) of persons with Hashimoto's disease may not have anti-TPO antibodies in their bloodstream There have been cases of negatively tested Hashimoto's hypothyroidism when thyroid antibodies production was restricted to the gland (Carbone *et al*., 2019).

Based on the results of table (3), the presence of both *H. pylori* antibodies used in this study was

significantly higher ($P<0.001$) among HT patients (94% for HP-IgG, 74% for anti-CagA) compared to the healthy subjects (17% for HP-IgG, 0% for anti-CagA). These findings indicate that there is an association between *H. pylori* infection and Hashimoto's disease, suggesting a role of the bacteria in the subsequent development of HT in Iraqi patients.

Consistence with our results, Al-Shaibani *et al* found that (94.07%) of HT patients from Baghdad city were seropositive to *H. pylori* IgG antibodies. Hamid (2017) revealed that *H. pylori* IgG was present in (57%) of HT patients in Baghdad city. Similar findings have been found by Arslan *et al*, and Elazaim *et al*, who concluded that there is an association between thyroid autoimmunity and *H. pylori* infection. This study was unable to clarify the *H. pylori-related* consequences that can cause thyroid autoimmunity, However, we agree with the opinion "The specific processes by which exposure to a microorganism induces more than one manifestation of autoimmunity are not well defined" (Hou *et al*.,2017; Hamid,2017). Furthermore, some of the thyroid proteins have recently been found to share putative conserved domains with numerous H. pylori antigens, hence *H. pylori* infection could induce HT disease through an increased inflammatory status and molecular mimicry (Figura *et al*.,2020).

The results of tables (4 and 5) showed that HT patients with positive results to HP-IgG and/or anti-CagA have significantly increased the serum levels of TSH and thyroid autoantibodies (anti-TPO and anti-Tg). The elevated levels of TSH, anti-TPO and anti-Tg especially in HT patients infected with CagA expressing strains of *H. pylori* could be due to the inflammatory response caused by *H. pylori* infection, which in turn increases the cytokines expression leading to increased infiltration of thy thyroid by the effector lymphocytes (due to the molecular mimicry). This could subsequently damage the thyroid tissues, thus increasing the disorder's progression. Our results were consistent and were confirmed by a local study (Aboud, 2011) in addition to other regional and international studies (Aghili *et al*.,2013; Shi *et al*.,2013; Korani *et al*.,2016). Otherwise, Bassi et al. concluded that the correlation between *H. pylori* and autoimmune thyroid diseases was found only with Graves's disease and not Hashimoto's. Also, Shmuely et al. couldn't clarify the importance of *H. pylori* infection in women suffering from Hashimoto's disease. We think that several factors may explain these variations in the results including the study population, size, area of study, methodology used for the diagnosis of *H. pylori* infection, types of tests used in the statistical analysis, and finally the genetic and environmental factors related to each study. However, the current study has several limitations including small sample size, and the dependence on serological methods only to diagnose *H. pylori* infection. So, more studies on the molecular level are badly required.

10. Conclusion

According to the results of the present study, it can be concluded that there is a relationship between *H. pylori* infection and the development of Hashimoto's thyroiditis in Iraqi patients. *H. pylori* infection, especially CagA expressing strains, could be a risk factor for the development of autoimmune hypothyroidism and to a lesser extent its progression increases the concentration of thyroid antibodies and TSH, which in turn leads to decreased levels of the thyroid hormones and worsening of the disease, thus requesting antibiotic therapy to eradicate the bacterial infection. Accordingly, it is reasonable to recommend the addition of thyroid antibodies (anti-TPO and anti-Tg) to the list of the thyroid function test and to examine the correlation between H. pylori infection with Graves' disease. More studies are recommended to clarify the molecular mechanism underlying the association between *H. pylori* infection and HT disease.

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