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Incidence of Hashimoto's thyroiditis and its relationship to age, sex, smoking and blood groups

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Abstract. This study was aimed to demonstrate the incidence of Hashimoto's disease in Iraqi patients and its correlation to some of the sociodemographic features. The research groups involved (50 patients with Hashimoto's disease and 50 healthy subjects). All of them were subjected to the estimation of levels of free triiodothyronine (FT3), free thyroxine (FT4), TSH, anti-thyroid peroxidase (anti-TPO), and anti-thyroglobulin (anti-Tg). The results demonstrated that there is a dramatic increasing in the occurrence of HT in the older patients. The age group (41-50 years old) was the most age group affected by Hashimoto's disease, followed by the age groups (31-40, 21-30, and 9-20 years old) respectively. A significant proportional correlation (R:0.952, P:0.024) was found between HT disease and aging. According to the gender, the results found that the vast majority (82%) of patients were females (P = 0.01). Also, smoking percent was (40%) of patients. The presence of autoimmune thyroid disease in one or more individuals within the family (family history) was also evaluated and the results found that (28%) of the Hashimoto's disease group have a positive family history to thyroid autoimmune diseases. It can be concluded from these results that females are more prone to developing Hashimoto's disease compared to males, in other words, the female gender is a risk factor for the occurrence of autoimmune hypothyroidism. Also, the negative impact of smoking and family history give an indication that these parameters are independent and don't associated with Hashimoto's disease at least in the current study.

Keywords: Hashimoto's disease, age, sex, smoking, anti-TPO, anti-Tg,

Introduction

Thyroid autoimmune diseases (AITDs) are many distinct clinical disorders, of which Hashimoto's hypothyroidism (HT) and Graves' hyperthyroidism were considered as the commonest disease (Caturegli *et al*.,2014). They reflect examples of autoimmune organic-specific diseases which are restricted to the thyroid gland (Casto *et al*.,2021). HT is highly abundant in female with an incidence ration of about 8:1. However, due to the laboratory findings of the thyroid autoantibodies in female, it appeared that about 10% of population are suffering from HT (Machała *et al*.,2019). In the pathogenesis, the thyroid antigens may be presented by dendritic cells as a 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, however they are genetically expressed and require an environmental trigger (Ragusa *et al*.,2019). Hashimoto's

thyroiditis can develop at any age, but it is most common in women between the ages of 30 and 60 years. The exact incidence of the condition is unknown, however it is thought to be similar to that of Grave's disease. According to researches, it's more common in areas with high iodine intake, and notably in people with genetic predispositions (Machała *et al*.,2019). Moreover, HT is more frequent in women than in men with an incidence of about 8 times higher in women than in men, however, according to the positive results of laboratory test in women for occurrence of autoantibodies for thyroid, it appeared that about 10% of population are present with HT (Vanderpump, 2019). The two environmental factors that have been researched more extensively in relation to HT are smoking and iodine. Smoking has a surprising positive effect on HT, despite the fact that it has a negative effect on Graves' illness. Tobacco cause lowers thyroid autoantibodies levels and decreases the likelihood of hypothyroidism in an uncertain mechanism (Ferrari *et al*.,2017). In this context, the current investigation aims is to demonstrate the incidence of Hashimoto's thyroiditis in Amara city patients. Also it can be evaluated if there is a correlation between the age, sex, family history and blood types with development of disease.

Materials and Working Methods

The present study enrolled 100 persons (from both genders) aging between (9-50 years old) during the period Dec. 2019 to Dec. 2020. Study participants are classified into two groups. The 1st group (50 patients) are attended to specified center of diabetes and endocrine glands diseases in Amara city with symptoms suspected to have Hashimoto thyroiditis. The second group (50 persons) are apparently healthy persons from comparable age and sex and considered as a control group. They all subjected to the serologic tests for the diagnosis of autoimmune thyroiditis. All of study participants have red and signed the patient consent form and the study has been accepted by Committee of Scientific Research Ethics / Amara Medical Institute.

Sample collection

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

Estimation of thyroid hormones levels in the samples

Blood concentrations of free triiodothyronine (fT3), free thyroxine (fT4) and thyroid stimulating hormone (TSH) were measured in the same day of blood collection by using electrochemiluminescence immunoassay method (Cobas, comp. Penzberg, Germany). As indicated in manufacturer's instructions, the results have been expressed in IU/mL.

Detection of thyroid auto-antibodies in the serum

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

Statistical analysis

The present study data were analysed using SPSS software package ver.23 (performed by IBM Co. USA). Independent T Test were used to test the significance between means. Odd ratio examination was also used to find out the relationship of *H. pylori* infection with HT disease. Statistical significant was detect when P value ≤ 0.05.

Results

This is a case-control investigation involved (50) HT patients and (50) healthy controls. All of the studied persons were subjected to the evaluation of serum FT3, FT4, TSH, anti-TPO, and anti-Tg. The current findings found a considerable elevation in serum TSH concentrations (P <0.001) in HT patients in comparison to the control group

as shown in the table(1). Serum FT4 and FT3 concentrations were also insignificantly elevated in the patients than in the healthy subjects.

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

Hormones	HT group Mean ± S.D	Control group Mean ± S.D	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. S.D: standard deviation**

The levels thyroid auto-antibodies (of both of anti – TPO and anti – Tg) were considerably elevated (P value < 0.001) in Hashimoto's patients with against control group, table (2).

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

Thyroid antibodies	HT patients Mean ± S.D	Control Mean ± S.D	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. S.D: standard deviation**

The effect of age on HT disease is described in the table (3) and figures (1 and 2). There is a dramatic increasing in the occurrence of HT in the older patients. The data of table (3) showed that the age group (41-50 years old) was the most age group affected by HT, followed by the age groups (31-40, 21-30, and 9-20 years old) respectively. A significant proportional correlation (R:0.952, P:0.024) was found between HT disease and aging, figure (1). This means that age may be considered as a risk factor for the development of HT disease.

Table 3: Distribution of the HT patients and healthy controls according to the age.

Age group (Year)	HT Patients		Control group	
	No	%	No	%
9 – 20	6	12%	14	28%
21 – 30	8	16%	17	34%
31 – 40	13	26%	7	14%
41 – 50	23	46%	12	24%

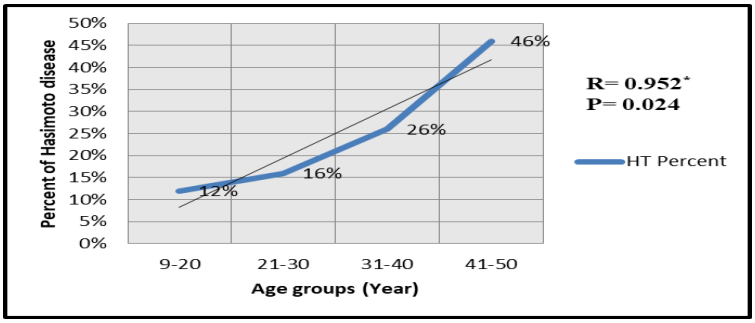

Figure 1: Pearson's correlation test between HT disease and age groups.

The results, of the table (4) describing the distribution of HT patients and healthy, are controlled according to different clinical features. In relation to the gender, it is clear that the vast majority of HT patients were females (82%) the results is highly significant (P = 0.01). In relation to the effects of smoking, the results found that smoking was non-significantly influencing (40%) of HT group. The presence of autoimmune thyroid disease in one or more individuals within the family (family history) was also evaluated and the results found that (28%) of the HT group have a positive family history to thyroid autoimmune diseases. It can be assumed from these results that females are more prone to developing HT disease compared to males, in other words, the female gender is a risk factor for the occurrence of autoimmune hypothyroidism. Also, the negative impact of smoking and family history give an indication that these parameters are independent and don't associated with HT at least in the current study.

Table 4: Differences in sex, smoking status, and family history in HT patients and healthy controls.

Gender	HT		Control		OR	95% CI:	P value
	No	%	No	%			
Male	9	18%	25	50%	0.2195	0.0884 to 0.5453	0.001**
Female	41	82%	25	50%			
Smoking							
Smokers	20	40%	25	50%	0.6667	0.3019 to 1.4721	0.3157
Non-smokers	30	60%	25	50%			
Family history							
FH +	14	28%	0	0%	40.1233	2.3181 to 694.4898	0.011**
FH -	36	72%	50	100%			

**Results are significant at 0.01 level. OR: odd ratio. CI: Confidence Interval. FH: Family History.

In the current study, blood grouping and Rh factor were also evaluated, and the results revealed, as shown in the tables (5 and 6), that (52%) of HT patients were of the blood type (O), (26%) of HT patients were of the blood type (A), and (14%, and 8%) of HT patients were of the blood types (B and AB) respectively. There was no significant association between HT disease and any other blood group compared to the healthy controls. The results also found, as seen in the table (6), that Rh factor was expressed in (94%) of HT patients compared to (98%) of the healthy controls without statistical significant differences (P= 0.33). This means that people with type O blood may be at high risk of developing autoimmune hypothyroidism, while those with type AB blood may be protected against such a disorder.

Table 5: Differences in blood groups among HT patients and healthy control.

Blood Groups	HT patients		Healthy controls		OR	95% CI	P value
	No	%	No	%			
A	13	26%	17	34%	0.682	0.2883 to 1.6137	0.383
B	7	14%	11	22%	0.5772	0.2036 to 1.6363	0.301
O	26	52%	18	36%	1.9259	0.8647 to 4.2897	0.108

AB	4	8%	4	8%	1.00	0.2320 to 4.3098	1.00

OR: odd ratio. 95%CI: Confidence Interval.

Table 6: Differences in the Rh factor in HT patients and healthy controls.

Rh Factor	HT patients		Healthy Control		OR	95% CI:	P value
	No	%	No	%			
Rh+	47	94%	49	98%	0.3197	0.0321 to 3.1837	0.33
Rh-	3	6%	1	2%	3.1277	0.3141 to 31.1434	0.33

OR: odd ratio. 95%CI: Confidence Interval.

Our results found that serum concentrations of TSH and thyroid auto-antibodies were considerably increased in Hashimoto's patients when comparing to normal healthy control. Also, thyroid hormones (FT3 and FT4) were elevated in patients but this elevation was not significant. These results could considered as an indication for the development of sub-clinical hypothyroidism which is distinct by higher level of elevated TSH and natural concentrations of thyroid hormones FT3 and FT4 (Machała *et al*.,2019).

For the diagnosis of HT, the current study was dependent on the estimation of blood concentrations of anti-TPO and anti-Tg antibodies and the results found , as shown in the table (2), that the concentrations of these antibodies were higher considerably (P<0.001) in the patients when compared to the normal healthy persons. Elevated concentrations of the thyroid autoantibodies and TSH as well as normal levels of thyroid hormones (FT3 and FT4) given an indication of the development of subclinical HT disorder. Anti-TPO and anti-Tg antibodies are widely available and commonly used in clinical diagnostic laboratories of HT disease (Congi and Chiovato, 2013). These antibodies are the major anti-thyroid antibody 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 concentration anti-Tg and anti-TPO in the blood can be used as markers for early prediction of the development of thyroid autoimmunity. They also recommend adding these tests in the same list of thyroid function test 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).

From the results of table (3), it's clear that aging are highly correlated with the development of Hashimoto's disease (P=0.024). Several Iraqi studies have found closely related results: Al- Mofarji (2013) found that HT disorder was most abundant in patients aging between (30-49 years), Alwayly (2016), stated that HT percent was highest among patients from the age group (40-49 years), Amin *et al*,(2018) recorded highest frequency of autoimmune thyroiditis in patients of ages between (31-40 year). It is indicated by several epidemiological global studies that HT may occur at any age of the human life but it is largely increased in patients aging from 30 to 60 years old (Wiersinga, 2016; Barbesino,2019). Generally, the exact incidence of HT is unclear but it may be comparable to that of Grave's disease. However, HT is more prevalent in areas with high iodine intake and exclusively in people with genetic predisposition (Machała *et al*.,2019).

In relation to the elevated occurrence of HT in females recorded in this study, Hashimoto disease was also higher in females (42.26%, 86.7%, and 95.7%) than in males (4.09%, 13.3% and 4.3%) as recorded by Al- Mofarji (2013), Al-Fatlawi (2014), and Alwayly (2016) respectively. Globally, results of different epidemiological studies revealed that there is a strong female preponderance to be affected by autoimmune thyroid diseases for example Pirahanchi and colleges found that HT are about ten times higher in females than in males (Pirahanchi *et al*.,2021). Similar results were found by (Castello, and Caputo, 2019; Calcaterra *et al*.,2020). The female dominance of Hashimoto's disease may be interpreted by the loss of self-tolerance to the X-linked antigens. There is emerging evidence shown that skewed XCI (deactivation of the chromosome X in about 80% of cells) which is considered as a risk factor for developing AITD in females (Simmonds *et al*.,2014; Effraimidis and Wiersinga, 2014). Furthermore,

the role of estrogen-induced immune response has also been suggested because estrogen making women more sensitive to autoimmunity throw the induction of pro-inflammatory response (Desai *et al*.,2019).

From the results presented in the table (4), smoking appears to be affecting (40%) of HT patients. There is no local study that considered the impact of smoking on the thyroid autoimmune disease especially Hashimoto and Graves' diseases. International studies have noted that smoking was associated with decreased prevalence of thyroid antibodies compared to the non-smokers (Strieder *et al*.,2003; Belin *et al*.,2004). Other studies however, did not confirm this data (Fisher *et al*.,1997; Knudsen *et al*.,2002). In this context, Åsvol *et al*,(2007) observed that current smokers have a lower frequency of subclinical hypothyroidism than never smokers in the population-based investigation. In another population based study, Carlé *et al*,(2012) concluded that the risk of being diagnosed with overt autoimmune hypothyroidism is 6-fold higher in the first two years after quitting smoking. On the other hand, current smoking have been found to increase by about two-fold the risk of Graves' hyperthyroidism with a dose dependent effect, which was more pronounced in women than in men, and disappears a few years after cessation of smoking (Wiersinga, 2013). Tobacco's components, such as nicotine, thiocyanate, and benzpyrene, have been related to thyroid hormone production suppression. Smoking also suppresses the production of TPO-Ab, which protects against the development of chronic lymphocytic thyroiditis and, as a result, an increased TSH to some extent. This explanation could explain why smoking has a bad influence on thyroid autoimmunity (Krassas, and Wiersinga, 2006).

In terms of AITD patients' family history, the present study discovered that (26%) of HT has a family history of the disease. According to the results of Al-Fatlawi study, 40% of HT patients and 37% of GD patients had relatives with AITD (Al-Fatlawi,2014). Familial studies have found that first-degree relatives had a higher disease prevalence than second-degree relatives over the world (Manji *et al*.,2006; Leung,2017). Thyroid autoantibodies are found in higher concentrations in the blood of those whose parents have Graves or Hashimoto disease (Jonsdottir *et al*.,2017). Graves' illness demonstrated a lower concordant familial connection than Hashimoto thyroiditis (Thomsen *et al*.,2020). Members of families with other autoimmune disorders, such as celiac disease, are at a higher chance of acquiring AITD (Wiersinga,2018). It has been established that thyroid autoantibodies and celiac disease in siblings have a positive connection (Emilsson *et al*.,2015). Because multiplex families (parents and siblings) have such high risks, penetratory genes with relatively high penetrance have a role (Thomsen *et al*.,2020).

Antigens of the blood group are fundamental molecules, and their phenotypic expression has been linked to a variety of disorders, including autoimmune diseases. Since Aird and coworkers discovered the link between the (A) blood group and gastric cancer, the relationship between blood groups and numerous diseases has piqued interest (Aird *et al*.,1953). The only local study which estimate the blood group variation and Graves' disease has revealed that significant incidence (43%) of the Graves disorder was seen in patients from blood type O (Aljorany & Abdal 2013). Similarly, in a Turkish study, the blood type O was significantly associated with HT disease (Dağdeviren *et al*.,2019). The high rate of HT in patients with blood type O could be due to the fact that blood type O is the most common blood group in Iraq, hence most of the patients will be of this blood type (Saleh, and Abood, 2016). Another possibility is that there is a link between particular blood groups and the development of autoimmunity, which was not discovered in the current study. More research is needed to confirm which factors were significant.

Conclusion

From the results above, it is clear that there is a relationship between the development of Hashimoto's thyroiditis and age in Iraqi patients. Hashimoto's thyroiditis also, were increased significantly in female gender compared suggesting a role of the reproductive hormones as well as skewed X chromosome. Smoking was found to decrease the incidence of Hashimoto's disease in un clear mechanism yet and needed for further specific investigation. The current study is the first one in Iraq investigate the correlations between blood types and Hashimoto's disease and found no relationship between blood antigens and Rh factor with the occurrence of Hashimoto's thyroiditis which is applied on Iraqi patients. It can be assumed that aging and female sex may be a risk factors for Hashimoto's thyroiditis while smoking exhibit an opposite effects. More detailed studies are needed to confirm the above mentioned results.

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