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Article

Effect of parasite infection on IgE, Anti-TG, Anti-TPO of thyroiditis patients

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ABSTRACT

This study was conducted to investigate parasitic infection's effect on the thyroid gland's functions by hypo- or hyperthyroidism and inflammation by measuring some physiological and immunological indicators. 130 blood and stool samples were collected from both genders, including 80 samples from people with thyroid disorders and 50 samples from healthy people. Anti-TG, anti-TPO and IgE were estimated by using the Cobas e411 device. The general stool examination was carried out to confirm the presence of a parasitic infection and determine the type of parasites. The type of parasites found the most affected were *Entamoeba histolytica*, *Giardia lamblia*, *Blastocystis hominis*, and the last parasite, *Cryptosporidium*. Anti-TG, anti-TPO, and IgE immunoassays significantly increased hypo and hyperthyroidism cases compared to the control. The increase was higher in parasitic-infected patients than in non-infected patients compared to the control. Results of the infected were as follows: (94.41, 27.96, 361.44) IU/ml and (54.13, 18.48, 308.18) IU/ml, for non-infected (55.46, 18.48, 149.67) IU/ml and (89.56, 20.80, 90.67) IU/ml. Compared to the control (22.3, 7.05, 62.93) IU/ml, respectively. Conclusion: This study showed that infection with a parasite affected the performance of the thyroid gland.

Keywords: Parasites; Thyroid Disorders; Anti-TG; Anti-TPO; IgE.

INTRODUCTION

Parasites are microscopic creatures that live in or on another organism, receiving their food from the host and inflicting harm [1]. The parasite can attack different organs, for example, the thyroid gland, which is one of the most important endocrine glands in the body, as its hormones (T4 and T3) have an important role in regulating the body's basic metabolic rate, the generation of heat, contribute to fat metabolism by stimulating lipolysis, and production fatty acids as an energy source [2].

The thyroid is a bi-lobed gland, often described as resembling a butterfly or bow tie. It is positioned in the neck, between the Cervical 5 (C5) and thoracic 1 (T1) vertebrae, just below the thyroid cartilage (Adam's apple) of the larynx [3], which are connected by a small piece of tissue called the isthmus; around half of the individuals also possess an additional third lobe, usually triangular shaped and known as the pyramidal lobe [4,5]. Thyroxine (T4) and triiodothyronine (T3) are classical thyroid hormones with relatively well-understood actions. In contrast, the physiological role of thyroid hormone metabolites, also circulating in the blood, is less well characterized [6], T3 and T4 are synthesized from iodine and tyrosine, they elevate optimal growth, development, function and conservation of all body tissues.

The synthesis and secretion of these hormones affect a hormone released via the pituitary gland called thyroid–Stimulating Hormone (TSH). This gland also produces calcitonin, which plays a role in calcium homeostasis [7]. Anti-Tg and Anti-TPO were introduced to assess the autoimmunity of thyroid disorder basis. Recent and past investigations have seen the usage of anti-TG and anti-TPO, in combination with thyroid hormones, to evaluate the thyroid status of suspected individuals and to manage treatment regimens [8]. In a recent study, it was discovered that anti-TPO antibodies were increased in people who were diagnosed with Hashimoto's thyroiditis and infected with *Blastocystis hominis*. The treatment of *B. hominis* infection in these patients resulted in a decrease in TSH and anti-TPO [9]. This study aimed to study the relationship between parasitic infection and thyroiditis by determining the type of thyroiditis (hyperthyroidism or hypothyroidism) by measurement of T3, T4 and TSH hormones. Investigating the types of parasites present in patients with thyroiditis. Study the effect of parasites in patients suffering from thyroiditis (hypothyroidism or hyperthyroidism) on parameters Anti-TG, Anti-TPOand, and Total IgE and compare them with healthy people and patients suffering from thyroiditis without parasite infection.

MATERIALS AND METHODS

Study group

The patients were selected according to their signs of thyroid problems that were evaluated by the medical consultation staff and, in most cases, included such as diarrhea, mood changes, anger, anxiety, depression, weight loss, weight gain, oily skin, dry skin, sensitivity to temperature changes, and others. The blood samples were collected from 80 Iraqi patients with a problem with the thyroid gland and 50 healthy considered controls from both genders, aged (20-50 years).

General stool examination (GSE)

Macroscopic examination

This included examining stool samples with the naked eye to detect the physiological characteristics (color, consistency, odor, blood, mucus, and to see the adult worms if present).

Microscopic examination

Wet mount method

This examination was based on using the wet mounts method of stool sample exam according to Kukaschke *et al*. and WHO, [10,11].

Staining with permanent stains

According to Garcia, [12] staining of slides was performed. The staining with Giemsa stain was used to detect *Entamoeba histolytica* and other parasites. Staining with safranin stain to detect *Blastocystis hominis*. Staining with meth-

ylene blue to detect *Giardia lamblia* (trophozoite of parasite appears in dark blue). Staining with Ziehl-neelsen stain to detect *Cryptosporidium parvum* (Oocyct of parasite appears in red).

Measurement of IgE, Anti-TG and Anti-TPO hormones

The level of total IgE in serum was done according to the manufacturer information company of the Cobas-Roche/ Germany kit(04827031, 2020). The level of anti-TG in serum was estimated according to the manufacturer information company of the Cobas-Roche/ Germany kit(06368697, 2017). The level of Anti-TPO in serum was done according to the manufacturer information company of the Cobas-Roche/ Germany kit (06368590, 2017). The same company manufactures the kit used in the device Cobas e411, and the method to work is the automatic system.

RESULTS

Distribution groups of the study

The total subjects in this study included 130 blood and stool samples: 80 samples from people suffering from thyroid disorders and 50 samples from healthy people of both sexes. Then they were distributed according to hormonal tests (T3, T4 and TSH) for hypothyroidism, hyperthyroidism and normal; after stool examining the stool, the total samples were divided into hypothyroidism with the parasite and without parasite, hyperthyroidism with the parasite and parasites.

Percentage rate of parasite infection according to age groups

The results showed that the highest infection with parasites was in the age group 20-30 years, and in hyperthyroid patients, the highest percentage than hypothyroidism, and the lowest rate of infection of parasites in the age group 41-50, as well as the Percentage rate of infection in hyperthyroid patients more than hypothyroidism as shown in Table 1.

Age ranges year	**Tested groups N(%)**					
	hyper w.p	Hyper w.o.p	Total	hypo w.p	hypo w.o.p	Total
20-30	7(19.5)	10(27.7)	17(47.2)	11(25)	4(9.1)	15(34.1)
31-40	4(11.1)	3(8.3)	7(19.5)	10(22.7)	5(11.4)	15(34.1)
41-50	5(13.9)	7(19.5)	12(33.3)	7(15.9)	7(15.9)	14(31.8)
Total	16(44.5)	20(55.5)	36(100)	28(63.6)	16(36.4)	44(100)

***hyper: hyperthyroidism, *hypothyroidism, *w.p: with parasites, *w.o.p: without parasites,**
***N: number.**

Table 1. Percentage rate (%) of parasite infection according to age groups.

Numbers and types of parasites in study groups

After examining the stool by using some special stains to diagnose parasites, the present four types of parasites were detected in this study: *Entamoeba histolytica, Giardia lamblia, Blastocystis hominis and Cryptosporidium parvum.*The rate of parasite infection with these parasites in hypothyroidism is shown in Table 2.

	E.histolytica	***G.lamblia***	***B.hominis***	***C.parvum***	**Total**
Hypothyroidism	19	16	9	1	45
Hyperthyroidism	7	5	4	1	17
Control	1	3	2	0	6

Table 2. Numbers and types of parasites in study groups.

Effect of parasitic infection in thyroiditis patients on some immune factors

This study measured some immunological indicators (IgE, anti-TG, and anti-TPO) In the sera of patients suffering from thyroiditis associated with parasitic infections. The study showed in Table 3 a significant increase in all immunological indicators in cases of hypothyroidism than hyperthyroidism compared to control. However, this increase was more in patients with parasitic infection. The results in Table 4 showed that *B. hominis* had the most effect on the antibody IgE than *E. histolytica* and *G. lamblia*, as *E. histolytica* more effect on Anti-TG than *G. lamblia* and *B. hominis* as the result were (94.09, 79.88, 60.22 IU/ml) respectively, while Anti-TPO the result showed that *G. lamblia* more effect than *E. histolytica* and *B. hominis*.

DISCUSSION

According to the group, Jaffar *et al*. [13] concluded that the prevalence of parasitic infection was significantly higher among younger age groups than older age groups. The reason may lead to poor conditions of hygiene, such as eating different foods from a variety of sources outside the home, which gives a greater chance of diarrhea [14], or because parasitic sicknesses might be a reason for other medical issues, for example, a ruptured appendix, cholecystitis, and intestinal obstacle [15]. With age, the thyroid gland suffers from progressive fibrosis and atrophy, which leads to a decrease in the size of the thyroid gland, making it difficult to touch. The prevalence of autoantibodies increases with age and may be partly responsible for anatomical changes in the thyroid gland [16]. Neoplastic disorders of thyroid and hypothyroidism are more common in the elderly, while hyperthyroidism is less common. This may explain the high prevalence 5.9 %) of hypothyroidism in patients. Also, the prevalence of neoplastic disorders, hypothyroidism and thyroid autoimmunity increases with age.

Tested groups	Immunological factors /Hypo			Immunological factors/Hyper		
Normal	ANTITG IU/mL	ANTITPO IU/mL	IgE ng/mL	ANTITG IU/mL	ANTITPO IU/mL	IgE ng/mL
Mean	C 22.38	C 7.05	B 62.93	C 22.38	B 7.05	C 62.93
±Std. Error of Mean	1.33	0.61	4.33	1.33	0.61	4.33
With Parasites	ANTITG	ANTITPO	IgE	ANTITG	ANTITPO	IGE
Mean	A 94.41	A 27.96	C 361.44	B 54.13	A 20.26	A 308.18
±Std. Error of Mean	13.55	2.82	13.28	14.82	3.42	17.19
WithoutParasites	ANTITG	ANTITPO	IgE	ANTITG	ANTITPO	IGE
Mean	B 89.56	B 20. 84	A 149.67	A 55.46	A 20.10	B 90.67
±Std. Error of Mean	18.05	2.88	7.07	19.89	2.99	2.44
P value	0.001	0.001	0.001	0.001	0.05	0.001
***hypo: hypothyroidism, *hyper: hyperthyroidism, *Std: standard deviation.**						
Data expressed as mean±Std. Error. LSD test was used to calculate the significant differences between the tested mean, the letters (A, B and C) LSD for columns represented the significant levels, The highly significant start of the letter (A) and decreasing with the last one. P value ≤ 0.05. Similar letters Mean there are no significant differences between tests mean.						

Table 3. Comparison of immunological tests between hypothyroidism and hyperthyroidism Patients.

On the other hand, hyperthyroidism is more common in the younger population [17]. The study conducted by Zaman *et al.* [18] showed that the prevalence of hypothyroidism increases with age, with a peak incidence between the ages of 30 and 50. Also, Kim [19] found that hypothyroidism increases in incidence and prevalence of age, particularly among the elderly. A local study by Salman *et al.* [20], who studied the occurrence of some intestinal parasites among diarrheic children, concluded that *E. histolytica* had a higher incidence than *G. lamblia* and *B. hominis*. Bazzaz *et al.* [21] studies about the prevalence of two gastrointestinal parasites, *Entamoeba histolytica* and *Giardia lamblia*, in Samarra City, concluded that *E. histolytica* had a higher incidence than *G. lamblia*. Hussein and Meerkhan [22] studied the incidence of intestinal parasites among children in Duhok City and concluded that *E. histolytica* had a higher incidence than *G. lamblia*, *Cryptosporidium* and *B. hominis*. Also, Jaffar *et al.* [13], who studied the incidence of intestinal parasitic infections among random samples in Wasit province, concluded that *E. Histolytica* was a higher incidence than *G. lamblia*, *Cryptosporidium* and *B. hominis*. Rhadi *et al.* [23] studied the prevalence of intestinal pathogenic parasites in Basrah City and concluded that *Cryptosporidium* had a higher incidence than *E. histolytica*, *G. lamblia* and *B. hominis*.

	Immunological tests		
Parasites types	**ANTITG IU/mL**	**ANTITPO IU/mL**	**IgE ng/mL**
***E. histolytica* Mean**	A 94.09	B 25.78	B 360.71
±Std. Error of Mean	13.57	3.10	12.24
***G. lamblia* Mean**	B 79.88	A 28.16	C 334.45
±Std. Error of Mean	15.80	2.90	17.00
***B. hominis* Mean**	C 60.22	C 24.24	A 403.33
±Std. Error of Mean	15.51	4.44	10.47
P value	0.001	0.03	0.0001
***Std: standard deviation.**			
Data expressed as mean±Std. Error. LSD test was used to calculate the significant differences between the tested mean, the letters (A, B and C) LSD for columns represented the significant levels, The highly significant start of the letter (A) and decreasing with the last one. P value ≤ 0.05. Similar letters Mean there are no significant differences between tests mean.			

Table 4. Effect of parasitic infection in thyroiditis patients on some immune factors.

Moreover, Kanabe and Darogha [24], who studied the epidemiology of Cryptosporidiosis among diarrheic children in Erbil, showed that *Cryptosporidium* had a higher incidence than *G. lamblia*, *B. hominis* and *E. histolytica*. Farhan, [25] and Al-Taei, [26], examined the general population, hospital patients and school children in urban and rural districts and found parasites infection are highly prevalent in all examined groups. The progress in establishing and developing a good water supply, electricity, paved roads and education led to the prevalence rates dropping for helminthiasis in 2002 but not for protozoan infections [27]. Rajič *et al.* [28] found that *B. hominis* infection in hypothyroidism patients affects thyroid hormones and noted that there is *G. Lamblia* infection, there was no direct effect on thyroid function. One of the main cause of thyroid disease is autoimmune thyroid disease, and anti-thyroid peroxidase (TPO) antibodies is a major marker of the condition; this antibody is especially helpful in the case of subclinical hypothyroidism in deciding the initiation of treatment and the duration of treatment, also in patients with subclinical hypothyroidism, presence of anti-TPO antibodies is associated with an increased risk of developing overt hypothyroidism [29,30]. Anti-TPO was considered to be a sensitive marker for autoimmune thyroid disease. The study performed in Norway revealed an association between anti-TPO levels and abnormally low and high TSH concentrations [31]. There was a strong relationship between thyroid diseases and IgE, and the specific IgE development against thyroid peroxidase (TPO), elevation of serum IgE among Thyroid-related antibodies are usually related to thyroid autoimmune diseases [32]. The study conducted by Vanderpump *et al.* [33] showed that anti-TPO was considered a sensitive marker for autoimmune thyroid disease. They revealed that hypothyroidism and hyperthyroidism were associated with positive anti-TPO. The study in Iraq noticed a higher level of anti-TPO in the hypothyroidism group [34]. Both Graves' hyper-

thyroidism and Hashimoto's thyroiditis are thyroid autoimmune diseases. Hashimoto's thyroiditis may occur after remission by anti-thyroid treatment in about 10 to 15% of Graves' hyperthyroidism cases. They assumed that it is due to the expansion of the immune response of autoantibodies that stimulate TSH receptor [35]. The production of specific antibodies to thyroid tissue is one of the most important features of Hashimoto's thyroiditis. In most Hashimoto's thyroiditis patients, there are specific autoantibodies for TG and TPO. In addition, the anti-TPO antibody assay is useful in predicting a condition of hypothyroidism [36,37]. Production of autoantibodies against TPO and TG causes lymphocytic infiltration and defects in thyroid cells; finally, hypothyroidism occurs. also, they suggested continuous monitoring of thyroid function in patients with Graves' disease, and the diagnosis of Hashimoto's disease (chronic thyroiditis) was based on clinical manifestation, high TSH level, positive thyroid peroxidase antibody, thyroglobulin antibody, and supported by positive results of fine needle aspiration biopsy [38]. El Din *et al.* [39] studies showed a statistically significant increase in the anti-TG level in patients with hypothyroidism and patients with hyperthyroidism compared with the control group. Similarly, a study in Saudia Arabia reported a statistically significant increase in anti-TG levels in the hypothyroid and hyperthyroid groups [40]. Another study in India reported a statistically significant increase in the anti-TG level in female patients with hypothyroidism when compared with control group [41].

Elevated IgE levels in several helminthic infections can also induce an antibody-dependent, cell-mediated cytotoxic response against helminthic parasites. IgE that binds to such parasites focuses on eosinophil targeting against the parasites. Once IgE is bound to the helminthic parasite and eosinophils bind to IgE, the eosinophil can degranulate against the parasite, IgE binds to the target parasite antigen, the eosinophil FcRe receptor binds to face of IgE, the eosinophil degranulates toward the parasite, and toxic products of the released eosinophil granules damage, destroy, or dislodge the parasite [42]. Long *et al*. [43] showed that *Blastocystis hominis* infection is associated with activation of immune cells and an increase in various types of interleukins. This may explain the effect of *B. hominis* on the IgE levels. A case report involving a 49-year-old man with Hashimoto's Thyroiditis shows that eradicating *Blastocystis hominis* resulted in a decrease in thyroid antibodies [28]. El-Zawawy *et al.* [44] showed that treating *B. hominis* infection in patients with Hashimoto's thyroiditis resulted in a significant reduction in TSH and anti-TPO, subsequently improving Hashimoto's thyroiditis.

CONCLUSIONS

In the current study, the younger age groups with thyroiditis are likelier to be infected with parasites. It showed the effect of parasites on the immune factors more than the physiological factors in thyroiditis patients. Parasite infection affected IgE more than anti-TG and anti-TPO in thyroiditis patients.

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