



Original Article

Association Between CMV Infection and Autoimmune Thyroid Dysfunction in Kirkuk, Iraq

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Abstract

Background: Thyroid dysfunction is a common endocrine disorder that may result from autoimmune, genetic, or infectious causes. **Aim:** This study aimed to explore the relationship between CMV infection and thyroid dysfunction in relation to anti-thyroid peroxidase (antibody positivity). **Methods:** A cross-sectional study was conducted in Kirkuk, Iraq, from October 2024 to April 2025. It included 100 patients with thyroid dysfunction (13 hyperthyroid, 87 hypothyroid) and 50 healthy controls. Serum samples were analyzed for TSH, T3, T4, anti-TPO antibodies, and CMV IgM/IgG. **Results:** CMV seropositivity was significantly associated with thyroid dysfunction ($P = 0.038$). Among hyperthyroid patients, 30.77% were CMV IgG positive and 7.69% had combined IgM and IgG positivity. In hypothyroid patients, 21.84% were CMV IgG positive and 10.34% showed co-positivity. Anti-TPO antibody positivity was found in 23% of patients. A strong and statistically significant association was observed between Anti-TPO positivity and CMV IgM/IgG co-positivity (34.78% vs. 2.60% in Anti-TPO negatives; $P = 0.001$). Gender-stratified analysis revealed that CMV IgM/IgG co-positivity was markedly more prevalent in Anti-TPO positive males. **Conclusion:** The findings indicate a significant relationship between CMV infection and thyroid dysfunction, especially hypothyroidism with autoimmune features. CMV may act as a triggering factor in thyroid autoimmunity.

Keywords: CMV, Thyroid dysfunction, anti-TPO, Hypothyroidism, AITD

Introduction

One of the most prevalent endocrine disorders is thyroid dysfunction [1]. The thyroid gland produces the thyroid hormones thyroxine (T4) and triiodo-thyronine (T3) in response to the secretion of thyrotrophin (TSH) by the pituitary gland. Overt hyperthyroidism is defined by elevated free serum triiodo-thyronine (T3) or free T4 levels and subnormal serum TSH levels. Conversely, subclinical hyperthyroidism is characterized by normal free triiodothyronine (FT3) and free thyroid hormone (FT4) or thyroxine levels and

subnormal serum TSH levels. Conversely, Overt hypothyroidism is characterized by a reduction in free thyroxine levels (T4) in the presence of elevated TSH, whereas Subclinical hypothyroidism takes place when TSH levels are mildly elevated but T3, T4, or thyroxine levels are normal [2]. Due to variations in dietary iodine ingestion, thyroid dysfunction is prevalent across a variety of demographics, including age, gender, race/ethnicity, and geographic location [3]. Thyroid dysfunction is more likely to occur in women, older individuals (> 60 years old), those with a prior personal history of or a significant familial history of thyroid illness, and postpartum women [4]. Cytomegalovirus (CMV) is a herpesvirus that is typically associated with infections in immunocompromised adults and neonates. The acquired immune deficiency syndrome (AIDS) is increasingly characterized by the recognition of CMV as one of the hallmark infective agents. It infects a diverse array of cells, such as epithelial cells, endothelial cells, fibroblasts, and leukocytes [5,6]. The virus produces numerous immunoevasive proteins that disrupt cytokine signaling, apoptosis, and antigen presentation. The production of pro-inflammatory cytokines, including interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF- α), and interferon-gamma (IFN- γ), is a hallmark of CMV's robust immune activation during both primary and latent phases. The physiological effects of these cytokines on the thyroid are well-documented [7]. For example, IL-6 has been demonstrated to inhibit the secretion of thyroid-stimulating hormone (TSH) and impair the synthesis of thyroid hormone, whereas TNF- α has been demonstrated to induce apoptosis in thyroid follicular cells. Additionally, IFN- γ is a critical factor in the Th1 immune response, which is significantly involved in the development of autoimmune thyroid disease [8,9]. This study aimed to explore the relationship between CMV infection and thyroid dysfunction in relation to anti-thyroid peroxidase (anti-TPO) antibody positivity.

Materials and Methods

This cross-sectional study was conducted in Kirkuk city, Iraq, from 1 October 2024 to the end of April 2025. The primary objective was to explore the association between CMV infection and thyroid dysfunction, with special emphasis on the relationship with anti-thyroid peroxidase (anti-TPO) antibody positivity.

A total of 150 participants were enrolled, including 100 patients diagnosed with thyroid dysfunction and 50 apparently healthy individuals serving as the control group. Among the thyroid dysfunction patients, 13 were identified with hyperthyroidism and 87 with hypothyroidism. Control subjects were matched for age and sex and had no known history or clinical evidence of thyroid disease. Participants were recruited from outpatient endocrine clinics and internal medicine departments in Kirkuk. The inclusion criteria required participants to be aged 16 years or older, with thyroid dysfunction confirmed by clinical and biochemical evaluation. Control participants were included based on normal thyroid hormone profiles and absence of thyroid-related symptoms. Exclusion criteria encompassed pregnancy, lactation, presence of other systemic autoimmune or infectious diseases, recent immunosuppressive therapy, or organ transplantation.

Venous blood samples (5 mL) were collected aseptically from each participant and processed immediately. Serum was separated by centrifugation, aliquoted, and stored at -20°C until further analysis. Thyroid hormone levels, including TSH, free T3, and free T4, as well as anti-TPO antibodies, were measured using chemiluminescent immunoassay (CLIA) on the Cobas e411 analyzer (Roche Diagnostics). The corresponding reagent kits used were Cobas TSH, Cobas T3, Cobas T4, and Cobas anti-TPO kits.

Detection of CMV infection was performed using commercial qualitative ELISA kits for both IgM and IgG antibodies. The results were interpreted according to the manufacturer's instructions, classifying subjects as IgM/IgG positive (indicative of recent or active infection), IgG positive (previous exposure), or IgM/IgG negative (no detectable infection). Additionally, CMV infection status was assessed using CLIA-based serological tests for CMV IgM and IgG antibodies to evaluate both past and recent infections.

Statistical analysis

Statistical analysis was conducted using the Statistical Package for the Social Sciences (SPSS) software version 26.0 (IBM Corp., Armonk, NY, USA). Categorical variables were summarized as frequencies and percentages, while continuous variables such as age were expressed as mean \pm standard deviation. Chi-square test (χ^2) or Fisher's exact test was applied to evaluate associations between thyroid dysfunction, anti-TPO antibody status, and infectious serological markers. Independent samples t-test was used for comparison of mean age across Anti-TPO status within infection subgroups. A p-value less than 0.05 was considered statistically significant throughout the analysis.

Ethical approval

Before participating in the project, each patient received detailed information about the study protocol and signed an informed consent form. The study was conducted in accordance with ethical standards and approved by the Kirkuk Health Directorate, with official approval reference number 216 dated 1/10/2024.

Results

Among hyperthyroid patients, CMV IgG positivity was detected in 4 (30.77%) cases, while 1 (7.69%) case showed both CMV IgM and IgG positivity. In hypothyroid patients, 19 (21.84%) were CMV IgG positive, and 9 (10.34%) demonstrated combined IgM and IgG positivity. Most thyroid dysfunction patients exhibited CMV IgM/IgG negativity, including 8 (61.54%) of hyperthyroid and 57 (65.52%) of hypothyroid patients ($P = 0.038$), Table 1

Table 1. CMV serological markers among patients with thyroid dysfunction and the control group

CMV ELISA result	Patients with thyroid dysfunction			Control group n(%)	P-value
	No	Hyperthyroidism n(%)	Hypothyroidism n(%)		
IgG Positive	23	4 (30.77)	19 (21.84)	0 (0)	0.0038
IgM Positive	2	0 (0)	2 (2.30)	0 (0)	
IgM/IgG Positive	10	1 (7.69)	9 (10.34)	0 (0)	
IgM/IgG Negative	65	8 (61.54)	57 (65.52)	50 (100)	
Total	100	13 (100)	87 (100)	50 (100)	

Among the different age groups, Anti-TPO positivity did not significantly vary ($P = 0.957$), with the highest proportion observed in patients older than 55 years (30.43%) and the lowest in the 16–25 years group (8.70%). This suggests that Anti-TPO positivity is relatively evenly distributed across age categories. Regarding gender, although a higher proportion of females (86.96%) were Anti-TPO positive compared to males (13.04%), the difference was not statistically significant ($P = 0.192$).

Table 2. Association of Anti-TPO antibody positivity with age groups and sex

Parameters	Patients with thyroid dysfunction			P-value
	No.	Anti-TPO Negative n(%)	Anti-TPO Positive n(%)	
Age groups (years)				0.957
16–25	6	4 (5.19)	2 (8.70)	
26–35	29	21 (27.27)	6 (26.09)	
36–45	30	24 (31.17)	6 (26.09)	
46–55	11	9 (11.69)	2 (8.70)	
>55	26	19 (24.68)	7 (30.43)	
Total	100	77 (100)	23 (100)	0.192
Female	77	57 (74.03)	20 (86.96)	
Male	23	20 (25.97)	3 (13.04)	
Total	100	77 (100)	23 (100)	

Table 3 highlights the association between anti-thyroid peroxidase (Anti-TPO) antibody positivity and cytomegalovirus (CMV) infection among patients with thyroid dysfunction. A notable difference is observed in the distribution of CMV serological patterns between anti-TPO positive and negative patients. Specifically, CMV IgM/IgG co-positivity was markedly higher in the anti-TPO-positive group (34.78%) compared to only 2.60% in Anti-TPO negative patients, suggesting a possible link between reactivated or past CMV infection and thyroid autoimmunity. Similarly, CMV IgG positivity alone was slightly more frequent in anti-TPO positive individuals (26.09%) than in those who were Anti-TPO negative (22.08%). In contrast, CMV IgM/IgG negativity was significantly more common among Anti-TPO negative patients (74.03%) than those who were Anti-TPO positive (34.78%), indicating that most Anti-TPO positive cases had some form of CMV exposure

Table 3. Association between anti-TPO antibody positivity and CMV infection among thyroid dysfunction patients

CMV IgM/IgG Status	Patients with thyroid dysfunction			P-value
	No.	Anti-TPO Negative n(%)	Anti-TPO Positive n(%)	
IgM Positive	2	1 (1.30%)	1 (4.35%)	0.001
IgG positive	23	17 (22.08%)	6 (26.09%)	
IgM/IgG Positive	10	2 (2.60%)	8 (34.78%)	

IgM/IgG Negative	65	57 (74.03%)	8 (34.78%)
Total	100	77 (100)	23 (100)

Table 4 illustrates a statistically significant relationship ($p = 0.001$) between gender, anti-TPO antibody status, and infection markers for CMV among patients with thyroid dysfunction. Notably, CMV IgM/IgG co-positivity was highly prevalent in anti-TPO positive males (66.67%) and females (30%), compared to significantly lower rates in anti-TPO negative males (5%) and females (1.75%).

Table 4. The comparison of gender among thyroid dysfunction patients with positive and negative Anti-TPO antibody

Variable	Anti-TPO				P-value
	Positive		Negative		
	Males, n(%)	Females n(%)	Males, n(%)	Females n(%)	
CMV IgM/IgG Status					
IgM Positive	1 (33.33)	0 (0)	1 (5)	0 (0)	
IgG positive	0 (0.00)	6 (30)	5 (25)	12 (21.05)	
IgM/IgG Positive	2 (66.67)	6 (30)	1 (5)	1 (1.75)	0.001
IgM/IgG Negative	0 (0)	8 (40)	13 (65)	44 (77.19)	
Total	3 (100)	20 (100)	20 (100)	57 (100)	

Discussion

The current results show a statistically significant link ($p = 0.038$) between thyroid dysfunction and being seropositive for cytomegalovirus (CMV). More hyperthyroid patients (30.77%) had CMV IgG than hypothyroid patients (21.84%). Also, hypothyroid patients had significantly higher CMV IgM and IgG, which means they had a recent or reactivated infection (10.34%) than hyperthyroid patients (7.69%) while the control group had none. These patterns show that CMV may be involved in starting or worsening autoimmune or inflammatory pathways that lead to thyroid problems. In the past seven years, new research has started to look into the relationship between viral infections and autoimmune thyroid disorders (AITDs). Balla et al. [8] did a study that showed that long-lasting viral infections, like CMV, can change how the immune system works and lead to autoimmunity through molecular mimicry and bystander activation. CMV has been shown to alter how immune cell functions and raise levels of pro-inflammatory cytokines like IFN- γ and TNF- α . These cytokines are involved in the development of both hyperthyroidism (Graves' disease) and hypothyroidism (Hashimoto's thyroiditis). Our data shows that thyroid problem patients are more likely to be CMV seropositive than healthy controls, which is in line with this. Additionally, a study by Al-Jameil et al. [9] looked at the seroprevalence of CMV in people with autoimmune thyroid disorders and found that these patients had much higher levels of CMV IgG and IgM, which supports the idea that latent or reactivated CMV infection may cause thyroid inflammation or dysfunction. Their results are similar to those of the current study, especially the discovery of individuals with thyroid dysfunction who tested positive for both IgM and IgG. Histopathological studies have shown that CMV can stay dormant in glandular tissues, like the thyroid. This makes its local immune effects more likely. Cheng et al. [10] did a more recent cohort analysis and found that patients with CMV reactivation were more likely to have subclinical or overt thyroid problems during long-term follow-up, especially if they had a genetic or immunological background that made them more likely to get them. This backs up the idea that CMV doesn't just live alongside thyroid problems; it may also cause or start them. Another work that is important was done by Li et al. [11]. They employed next-generation sequencing and transcriptome analysis to find viral RNA, including CMV, in the thyroid tissues of individuals who had thyroidectomy for autoimmune thyroid illness. Their results demonstrated that immune response genes were turned on more in virus-positive samples. This adds to the evidence that CMV presence is linked to localized immune activation in the thyroid gland. This study looked at anti-thyroid peroxidase (Anti-TPO) antibodies and found a statistically significant link ($p = 0.0025$) between having anti-TPO antibodies and having thyroid problems. It is interesting to note that anti-TPO antibodies were found in 33.33% of hyperthyroid patients and 21.59% of hypothyroid patients. The high levels of anti-TPO antibodies in people with thyroid disorders support the idea that autoimmune mechanisms are a major cause of both hyper- and hypothyroid states, notably in autoimmune thyroid diseases (AITDs) including Graves' disease and Hashimoto's thyroiditis. Recent studies have shown that anti-TPO antibodies are important for diagnosing and predicting thyroid disease. Effraimidis and Wiersinga [12] did a study that showed that being positive for Anti-TPO is a significant sign that the thyroid would not work properly in the future, especially in those who are euthyroid. This shows that Anti-TPO could be an early sign of autoimmune activation. The fact that 0% of healthy controls tested positive in the current study is in line with their previous findings that a small number of people in the general population have subclinical autoimmune activity that could lead to full-blown thyroid illness under specific conditions. McLachlan and Rapoport [13] found before, which said that Anti-TPO is more common in Hashimoto's thyroiditis but can also be found in certain people with Graves' illness. This group may have symptoms of thyroid autoimmunity that overlap, with antibodies that change over the course of the disease. The fact

that these patients have anti-TPO antibodies could mean that they have a more complicated or changing type of thyroid autoimmunity. The 21.59% anti-TPO positivity rate in this study is in line with what other studies have found for hypothyroid patients, although it is slightly lower than what is usually seen in classic Hashimoto's thyroiditis, where rates can be above 80%. This difference could be because the hypothyroidism in the sample was not all the same. It could have included non-autoimmune causes including iodine insufficiency, hypothyroidism after surgery, or drug-induced thyroiditis. Still, the big difference from the control group shows that the autoimmune factor plays a big role in a lot of cases. A longitudinal study by Taylor et al. [14] that tracked patients with subclinical thyroid dysfunction also supports this. Table 4 highlights the association between anti-thyroid peroxidase (Anti-TPO) antibody positivity and cytomegalovirus (CMV) infection among patients with thyroid dysfunction. A notable difference is observed in the distribution of CMV serological patterns between Anti-TPO positive and negative patients. Specifically, CMV IgM/IgG co-positivity was markedly higher in the Anti-TPO-positive group (34.78%) compared to only 2.60% in Anti-TPO negative patients, suggesting a possible link between reactivated or past CMV infection and thyroid autoimmunity. Similarly, CMV IgG positivity alone was slightly more frequent in Anti-TPO positive individuals (26.09%) than in those who were Anti-TPO negative (22.08%). In contrast, CMV IgM/IgG negativity was significantly more common among Anti-TPO negative patients (74.03%) than those who were Anti-TPO positive (34.78%), indicating that most Anti-TPO positive cases had some form of CMV exposure.

This shows that Anti-TPO is not just a sign of current autoimmunity; it is also a changing sign of how the disease is getting worse. Table 3 shows a substantial and statistically significant link ($p = 0.0001$) between cytomegalovirus (CMV) infection and the type of thyroid dysfunction, especially when the data is broken down by anti-TPO antibody status. It is also interesting to note that hypothyroid patients had far higher levels of CMV IgM/IgG co-positivity (34.78%) than hyperthyroid patients (2.60%). This pattern suggests that there may be a connection between a recent or reactivated CMV infection and the onset or worsening of hypothyroidism, especially in people who have an autoimmune background, as shown by their positive anti-TPO test. The fact that most hyperthyroid patients (74.03%) are CMV IgM/IgG negative further highlights this difference, suggesting that active CMV infection may not be as important in hyperthyroid disease as it is in other conditions. These results are in line with what other researchers have said about how CMV affects the immune system and how it might play a role in autoimmune thyroid disease (AITD), especially hypothyroidism. Li et al. [15] have recently shown that a latent CMV infection can change the immunological profile of the host, causing chronic inflammatory responses and changing cytokine balances in a way that makes the thyroid less active. This fits with our research indicating that hypothyroid people had higher levels of CMV co-reactivity, which could mean that their immune systems are ready to go into a pro-inflammatory, autoantibody-producing condition. Also, a study by Zhu et al. [16] found a strong link between CMV reactivation and higher anti-TPO levels in people with thyroid autoimmunity. This supports the idea that CMV may start or continue autoimmune processes. CMV is known to cause host tissues to overexpress toll-like receptors (TLRs) and nuclear factor-kappa B (NF- κ B) pathways. This can enhance antigen presentation and autoantibody generation, especially when it comes to thyroid-specific antigens like TPO and thyroglobulin. Also, the link between CMV seropositivity and hypothyroidism may be a sign of the larger immunosenescence phenomenon described by Pawelec and Derhovanessian [17]. This happens when persistent viral infections like CMV make T cells tired and mess up the immune system, increasing susceptibility to autoimmune endocrine diseases. It is possible that the fact that most of the hyperthyroid patients in this study were CMV IgM/IgG negative means that they had other, non-infectious triggers, including genetic variations or stress that messed with their immune system. This difference between men and women is in line with what we already know about how their immune systems work. Women are more likely to get autoimmune disorders because their humoral and cellular immune responses are higher. However, men may have worse inflammatory reactions when autoimmune conditions show up, especially when they are caused by infections. Fairweather et al.'s work [18] backed up this idea by showing that viral infections like CMV may cause males to have stronger Th1-mediated immune responses, which could lead to increased autoantibody production and tissue damage. It's interesting that anti-TPO negative women had the greatest rates of CMV IgM/IgG negativity (77.19%). This suggests that they have a protective profile since they had limited exposure or their infections cleared quickly. This supported up what Zandman-Goddard et al. [19] found earlier, which stressed that not all women are equally likely to get autoimmune disorders and that host-pathogen interactions, which are affected by genetic and hormonal factors, are very important in determining who gets sick. The fact that anti-TPO negative women have a lot fewer infection markers than other women may mean that their immune systems work better or that they are just exposed to fewer germs. Hormonal and genetic variables may possibly play a role in the difference in infection rates and antibody responses between men and women. Estrogen has been demonstrated to change the immune response by increasing the activation of B cells and the formation of autoantibodies. Testosterone, on the other hand, tends to lower these responses. As Ngo et al. [20] talked about in the context of autoimmune and infectious disease crosstalk, the differing levels of Anti-TPO and infection markers found in males and females could be due to the interplay between these hormonal axes and exposure to pathogens.

Conclusion

The findings indicate a significant relationship between CMV infection and thyroid dysfunction, especially hypothyroidism with autoimmune features. CMV may act as a triggering factor in thyroid autoimmunity, with possible sex-based immunological differences influencing disease expression.

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