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THYROID AUTOANTIBODIES IN TYPE -1 DIABETES Mellitus Patients and their Correlation with Thyroid function and Tumor Necrosis Factor-Alpha

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ABSTRACT:

Type 1 Diabetes Mellitus (T1DM) is a complicated autoimmune disorder initiated by T-cell-mediated damage of pancreatic beta cells, resulting in insulin deficiency and the development of hyperglycaemia. This disease is most common in childhood and adolescence and frequently co-occurs with other autoimmune conditions like autoimmune thyroiditis. This work aimed to investigate thyroid autoantibodies and their correlation with thyroid functions and tumour necrosis factor-alpha (TNF-α) in T1DM patients. Eighty participants were enrolled in a case-control study, including sixty T1DM patients and twenty healthy controls. Peripheral blood specimens were taken from individuals with proven T1DM and healthy individuals (control group). Body mass index (BMI), fasting blood sugar (FBS), glycated hemoglobulin (HbA1c), thyroid-stimulating hormone (TSH), free triiodothyronine (FT3), free thyroxine (FT4), thyroid autoantibodies, including anti-thyroid peroxidase (anti-TPO) in addition to anti-thyroglobulin (anti-Tg), and TNF- α concentrations were evaluated in T1DM and control groups. Our findings revealed that thyroid autoantibodies were significantly more prevalent in T1DM patients, elevated TSH, and significantly higher TNF-α levels than in the control group, indicating an increased risk for inflammation based on cytokine levels. The current study proved that in T1DM patients' sera compared to the healthy control, thyroid autoantibodies and TNF-α levels were positively correlated. In conclusion, poor diabetes management and high TSH levels may indicate subclinical hypothyroidism, which impacts diabetes control. This is possibly linked to thyroid autoimmunity. Future research is needed to understand how TNF- α contributes to the progression of diabetes disease and its complications.

KEYWORDS: Type-1 Diabetes Mellitus, autoimmune thyroid antibodies, TNF-α.

1. INTRODUCTION

Diabetes is one of the medical issues with the fastest-rising prevalence in the twenty-first century. There were 463 million adult diabetics worldwide in 2019, and the International Diabetes Federation projected that number to increase to 700 million by 2045. (Atlas, 2015). Diabetes mellitus type 1 (T1DM) is a chronic condition that results in abnormal fat, carbohydrate, and protein metabolism because of a lack of insulin (Popławska-Kita et al., 2014). The thyroid hormone regulates metabolic functions necessary for healthy growth and development and adult metabolism. Increased thyroid hormone, also known as hyperthyroidism, results in weight loss, decreased cholesterol concentrations, and high lipolysis and gluconeogenesis (Mullur et al., 2014). In contrast, low thyroid hormone levels, or hypothyroidism, correlate with hypometabolism, distinguished by low resting energy expenditure, increased weight, raised cholesterol levels, minimal lipolysis, along with lowered gluconeogenesis (Brent,2012). Diabetes mellitus and thyroid diseases are closely related. Numerous studies have shown that thyroid disorders are more common in people with diabetes mellitus and vice versa (Biondi et al., 2019; Blaslov et al.,2020; Chaker et al.,2022). Moreover, through its effects on pancreatic beta-cell development and glucose metabolism in some organs, including the pancreas, skeletal muscles, adipose tissue, liver, digestive system, and central nervous system, thyroid hormone influences glucose homeostasis (Eom et al., 2022). Inflammatory cytokines like interleukin-6 (IL-6), IL-17, transforming growth factor-beta (TGF-), and C-reactive protein (CRP) are increased in DM patients. These elevated cytokines are crucial for the emergence and development of cardiovascular complications (Flores et al., 2004, Oghagbon et al., 2014). TNF-α is a cytokine that causes acute phase reactions and is involved in systemic inflammation. TNF-α may directly affect the insulin signaling pathway and cause insulin resistance, contributing to the pathogenesis of type 2 DM together with obesity (Hotamisligil et al., 1994, Qiao et al., 2017). In a recent study, Szabo et al. (2020) concluded that TNF- is a key player in the development and pathogenesis of T1DM and may serve as both an extra indicator of disease progression and a potential immunotherapeutic target. T1D is a highly heterogeneous condition affected by various variables, including ageing, genetic predisposition, and environmental interactions. Through interactions with immune cells, pancreatic cells also seem crucial in starting pathogenic processes. β-Cells appear to be more active participants in the disease's development than previously thought and are not merely passive targets (Li et al., 2021, Zajec et al., 2022).

The present work aimed to investigate serum levels of thyroid autoantibodies and their correlation with thyroid function and tumor necrosis factor-alpha (TNF-α) in T1DM patients compared to randomly selected healthy individuals.

2. MATERIALS AND METHODS

1.1 2.1. Study Subjects

Patients with T1DM aged (5-40 years) were eligible for this study compared with the age and sex matching control group of

* Corresponding author

healthy subjects. The demographic information, including; sex, age, height, weight, and family history, were included in a questionnaire filled by the participants and a consent form before taking the sample.

1.2 2.2. Sample collection

Peripheral blood samples were collected aseptically from sixty confirmed patients afflicted with T1DM from Ashti Hospital, Layla Qasim Health Center, Raparin Hospital, and Rzgary Hospital, Erbil, from November 2021 to February 2022. In addition, peripheral blood samples were drawn from twenty age-matched persons who were randomly selected as a healthy control group. Associated hospital staff and patient permission were obtained before sample collection, and the patient signed a consent form. A total of 5 mL of peripheral blood was collected from patients and healthy controls. 3 mL was placed in a serum gel tube for serum separation, and 2 ml was placed in an EDTA tube. The blood samples were centrifuged for 15 minutes at 3000 rpm after being allowed to clot for 30 minutes at room temperature. All prepared sera were kept at -20°C until further analysis for serological tests (Shani et al., 2012). The following formula was applied to estimate the Body Mass Index (BMI) kg/m2 of the study subjects, where a person's height is expressed in square meters, and their weight is represented in kilograms (kg). BMI = Weight (Kg)/ Height $(m)^2$

This work was performed under the guidelines of the ethical committee (issued from scientific research committee: Faculty of Science at Soran University: 1/1/178 on 24 of January 2021.

1.3 2.3. Haematological and serological tests

All samples were used to estimate the level of fasting blood sugar and glycated haemoglobin (HbA1c), which determine the quantity of blood sugar (glucose) that is linked to haemoglobin by COBAS C111 and TINA- QUANT are trademarks of Roche (Distribution in the USA by: Roche Diagnostics, Indianapolis, IN Made in Germany). Also, Anti-Tg, Anti-TPO, T3, T4, and TSH levels were estimated by COBAS E 411, and ELECSYS are trademarks of Roche (Distribution in the USA by: Diagnostics, Indianapolis, IN Made in Germany), (Cowie et al., 2010). The Cobas e 411 analyzer was used to measure anti-Tg, anti-TPO, T3, T4, and TSH. German company Roche Diagnostics GmbH, D-68298 Mannheim. While the serum levels of TNF- were assessed by enzyme-linked immunosorbent assay (ELISA) according to the manufacturing protocol of the kit. Human TNF-alpha ELISA Kit based on double biotin antibody sandwich technology (SUN LONG BIOTECH-China, Catalog Number. SL1761Hu) was used. The optical density (wavelength 450 nm) and standard concentrations were plotted on the y-axis and x-axis, respectively, to produce the standard curve. TNF-α concentrations in the tested samples were estimated using the standard curve (Shnawa et al., 2020, Hamad et al. 2021)

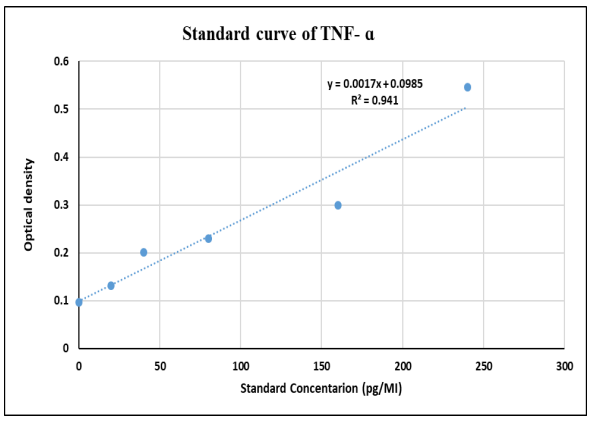


Figure 1. Standard curve for TNF-α determination by ELISA.

1.4 2.3 Statistical Analysis

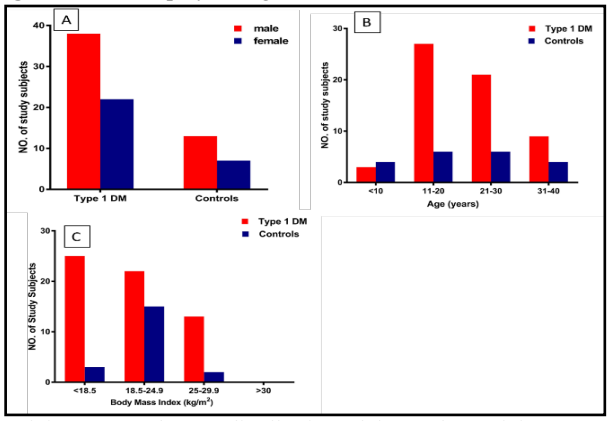
The initial normality and non-parametric test (Mann-Whitney Confidence Interval) were used to analyze the data statistically; the result is significant at < 0.05. Pearson's correlation was calculated to determine the tested parameters' correlation. Receiver operating characteristic (ROC) curves were generated, and the area under the curve (AUC) was evaluated to assess the diagnostic efficacy of each value in predicting the presence of TNF-α in T1DM. All statistics and graphics were created using Excel 2016 and GraphPad Prism 8.05.

3. RESULTS

3.1 Study Subject Demography

Demographic and clinical data containing age, gender, and BMI Kg/ m2 are presented in Figure 2. The results showed that the mean age for the T1DM and HC groups was 21.51 ± 0.848 and 21.55 ± 1.676, respectively. The present study showed that most T1DM patients were males counting 38 patients with a percentage of (63.3 %), while only 22 females (36.6 %) were females. In healthy control, females count as 7 and males as 13 as a normal population distribution. The distribution of age among T1DM patients showed that (41.7%) of T1DM patients were within the second group (11-20 years), which had the highest number, including 27 patients, followed by the third group (21-30) with 21 patients. In contrast, the lowest number of patients were found in the first group (< 10 years), including 3 (5%) patients. Regarding the BMI, the results showed that 36.7.0% (22) of T1DM patients were within the normal weight category, including (18.5-24.9 kg/m²), as well as 41.7% of patients, which included (25) were underweight compared to the healthy control group. Also, 21.6% (13) of T1DM showed to be overweight (25-29.9 kg/m²).

Figure. 2 A. Display the gender distribution of the research



participants. **B.** The age distribution of the study participants. **C.** Illustrates the study subjects' distribution according to their BMI.

3.2. Haematological and serological tests

The outcomes of this study demonstrated a significant rise in FBS levels in the T1DM patients compared to the control. ($p<0.05$), as illustrated in Table 1 and Figure 3, A. The findings revealed a noticeable rise in HbA1c levels in T1DM patients compared to the control ($p<0.05$), as shown in Figure 3, B.

The results revealed a non-significant decrease in level T3 in the T1DM patients compared to the healthy control, as illustrated in Figure 3 C. The results showed a non-significant reduction in the level of T4 in the T1DM group compared to the healthy control group, as illustrated in Figure 3, D. The results indicated a significant elevation in TSH levels in the type I diabetes patients compared to the healthy control (<0.0001), as illustrated in Figure 4 A.

Table 1. Concentrations of the study parameters in T1DM patients and the healthy control.

Parameters	T1DM No (60)	Control No (20)	P - Value
	Mean ± SD		
FT3 (pg/ml)	99.28 ±1.502	104.2 ± 2.98	0.2749
FT4 (pg/ml)	9.212 ± 0.1755	9.595 ± 0.2616	0.1021
TSH mlU/L	3.491 ± 0.3001	1.032 ± 0.1074	<0.0001
Anti -TPO lu/ml	73.93 ± 2.07	45.99 ± 2.773	<0.0001
Anti – Tg lu/ml	65.45 ± 2.252	33.46± 2.523	<0.0001
HbA1c %	9.244 ± 0.2576	5.623 ± 0.1107	<0.0001
Blood Sugar mg/dL	284.6 ± 18.24	89.75 ± 1.495	<0.0001
TNF-α (pg/mL)	28.14 ± 4.206	12.6 ± 2.451	0.0279

Table 2. Frequencies of thyroid autoantibodies in T1DM patients and control groups.

	Anti – TPO		Anti – Tg		Anti – TPO Anti – Tg
	+ ve No %	- ve No %	+ ve No %	- ve No %	+ ve No %
Type – I DM (N=60)	19 31.7	41 68.3	14 23.3	46 76.7	10 16.6
Control (N= 20)	0 0	20 100	0 0	20 100	0 0

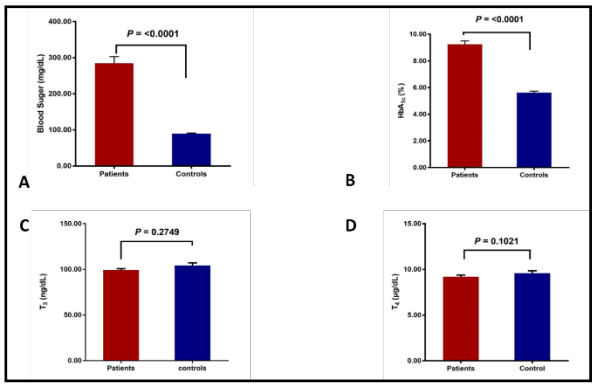


Figure 3.A. Fasting blood sugar for T1DM patients and healthy control**. B.** Serum levels of HbA1c of T1DM patients and control. **C.** Levels of T3 in T1DM patients in comparison to the control group. **D.** Levels of T4 in T1DM patients in comparison to the healthy control.

The results showed a significant elevation in anti-TPO antibody levels in the T1DM patients compared to the control ($p<0.0001$), as illustrated in Table 1 and Figure 4 B. Also, the present study observed a significant increase in anti-Tg antibodies level in T1DM patients compared to the control. ($p<0.0001$), as demonstrated in Figure 4 C.

Table 2 and Figure 5. show the frequency of thyroid antibodies in T1DM patients and control groups. The percentage of anti-TPO antibodies positivity was detected in 19 (31.7 %) out of 60 patients, and no positive value was seen in the control subjects out of 20 controls. Also, positive anti-Tg antibodies were found in 14 (23.3 %) out of 60 patients, and no positive value was detected among the controls.

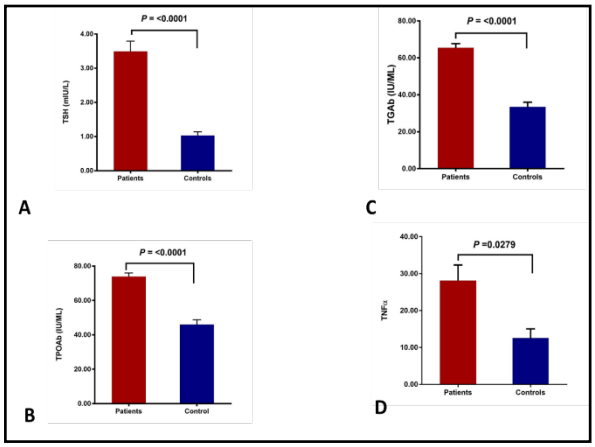


Figure 4. A. A significant elevation in TSH concentrations of T1DM patients compared to control. **B.** Shows a significant increase in anti-TPO antibody levels in the T1DM patients compared to the control. **C.** Significant increase of serum levels of anti-Tg antibody in T1DM patients compared to healthy control. **D.** The levels of TNF- α were significantly increased in T1DM patients in comparison to the control measured by the ELISA test.

The current study showed that The TNF- α was significantly increased in T1DM patients in comparison with the healthy control, as depicted in figure 4 D. The receiver operating characteristic (ROC) curve was performed to evaluate the best serum TNF- α cutoff value that can predict a diagnosis of T1DM. As depicted in Figure 5, the Cutoff value was 12 (pg/mL), sensitivity was 89 %, and specificity was 65%.

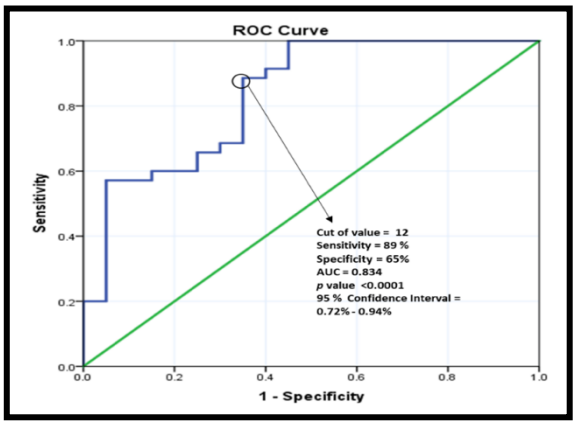


Figure 5. Receiver operator characteristic (ROC) curve to determine the best serum TNF- α (pg/mL) cutoff that can predict a diagnosis of T1DM disease**.**

Moreover, the seropositive and negative cases of T1DM patients and the control concerning anti-TPO levels showed that 31.7 % of patients were seropositive. The mean was 73.93 ± 2.07 ng/ ml leaving 68.3 % of patients as negative cases, the mean of anti-TPO levels in healthy control was 45.99 ± 2.773 ng/ml with a significant difference (<0.0001) between them, as in Table 2 and Figure 4B. Moreover,10 (16.6%) of the tested sera of patients were seropositive for anti-TPO and anti-Tg antibodies together.

In cases of anti-Tg antibodies, the results recorded that 23.3% of T1DM patients were seropositive and 76.7 % were seronegative, with a mean of 65.45 ± 2.252 and 33.46± 2.523 in patients and control, respectively, with a significant difference (<0.0001), as shown in Table 1 and Figure 6A.

Furthermore, a significant positive Pearson's correlation of a value of 0.2773 was calculated between TNF-α level anti-Tg antibodies, under p0.0320. Additionally, a positive Pearson's correlation between the anti-TPO titer and TNF-α was noticed as 0.282 with statistically significant (p=0.0383), as illustrated in Figure 6 C and 6 D.

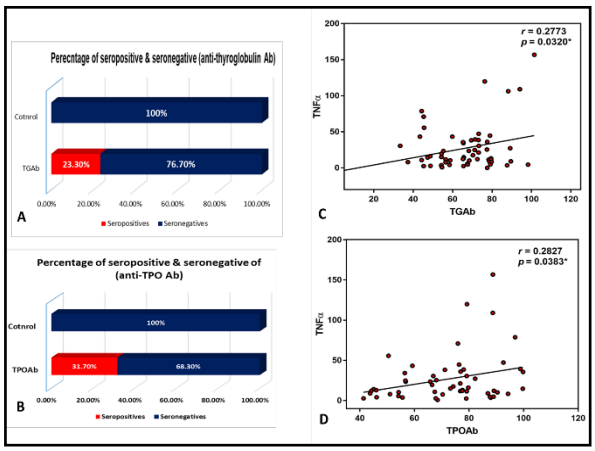


Figure 6. A&B Percentage of seropositive and seronegative T1DM patients and HC for anti- TPO and anti-Tg antibodies, anti-TPO and anti-Tg antibody levels were measured by ELISA. **C.** The correlation between TNF-α and anti-Tg levels in T1DM, the findings presented a positive correlation. **D.** Under p = 0.0383, there was a significant positive correlation between TNF- and anti-TPO levels in T1DM patients.

4. DISCUSSION

This study investigated thyroid function and thyroid autoantibodies levels in T1DM patients. The finding of this work revealed that T1DM disease was more distributed among males with the tested samples at 63.3% compared to females at 36.6%. Similar research showed that in Basrah, Iraq, T1DM was significantly higher in males than women (Almahfoodh et al., 2017). While in another study in Iraq, they showed that 52% of T1DM patients were females and 48%were males (Ridha and Al Zubaidi, 2019). The differences may be attributed to different sample sizes or environmental factors. T1DM and autoimmune thyroid disorder (AITD) are common autoimmune disorders that can coexist. Diabetes patients are generally at high risk for AITD. The pathogenesis of T1DM and AITD is thought to be influenced by a variety of nongenetic factors as well as a complex genetic basis (Li et al., 2020). Type I diabetes is an autoimmune disorder; it may be linked to other autoimmune conditions that, by interfering with an organ's normal function, can affect how well diabetes is managed (Oh et al., 2016). Frommer and Kahaly (2021) pointed out that the shared genetic background of these two diseases largely explains their close association. Additionally, it has been discovered that functional single nucleotide polymorphisms of numerous genes, including the tumour necrosis factor (TNF), the protein tyrosine phosphatase non-receptor type 22, the interleukin-2 receptor (IL2Ra), the cytotoxic T-lymphocyte-associated antigen, the cytotoxic T-lymphocyte-associated antigen, and vitamin D receptors are elaborate in immune control have been recognized to increase susceptibility to both T1D and AITD (Frommer and Kahaly, 2021). The current results indicated that 31.7% and 23.3% of T1DM patients had positive anti-TPO and anti-Tg antibodies, respectively.

Additionally, sera of T1DM patients showed significantly high levels of TSH and slightly non-significant decreases of T3 and T4 compared to the healthy control group. These findings are consistent with previous researchers who demonstrated high positive serum values of anti-TPO and anti-Tg in 150 Iraqi patients with Type-1 diabetes, which is associated with increased TSH and proposed an association between elevated TSH levels and the onset of hyperglycaemia (Ridha and Al Zubaidi, 2019; Milovanovic´ et al.,2022). Similarly, Ridha and Al Zubaidi (2019) emphasized that TSH levels were elevated in 16% of the studied T1DM patients, and all recorded positive anti-TPO and anti-Tg. This finding was also observed by Padberg et al., 2001), who concluded that Anti TPO is associated with high TSH levels in Hashimoto's thyroiditis patients .this fact was also confirmed recently by Li et al. (2020). They mentioned that both AITD and T1DM are common autoimmune disorders frequently found together, and patients with diabetes have a high risk of AITD. Moreover, Muhammed and Albustani (2018) found that the anti-TPO and anti-Tg thyroid autoantibodies and TSH were significantly positively correlated. Additionally, when serum TSH levels are abnormal and free T4 and free T3 levels are within the appropriate reference values, subclinical thyroid disease (SCTD) is present. In clinical practice, SCTD is more frequently diagnosed in young, middle-aged, and elderly patients (Biondi and Cooper, 2008). Depending on the patients' TSH, T3, and T4 results and the healthy controls, the patients in our study seem to categorize as subclinical hypothyroid cases (Blaslov et al., 2020). Padberg et al.(2001) also mentioned that the Anti-TPO is associated with more increase in TSH concentration than anti-Tg. In patients with Type-1 diabetes, thyroid autoantibodies are strongly correlated with the likelihood of developing thyroid dysfunction in the future, depending on autoantibody presence and TSH (Denzer et al., 2013). In uncontrolled diabetics, the TSH response to thyrotropin-releasing hormone administration

was found to be noticeably diminished. The inverse relationship between fasting plasma glucose and HbA1c levels and thyrotropin peak was observed (Junik et al., 2006). The current study's finding revealed that anti-TPO and anti-Tg levels significantly increased in T1DM patients is in line with several earlier results in this area (Kakleas et al., 2009, Shiva and Behbahani, 2009, Kedari, 2010, Ardestani et al., 2011). Moreover, Nederstigt et al. (2019) concluded in a meta-analysis study the prevalence of the antibody-mediated autoimmune disease is elevated in the sera of T1DM patients. Particularly hypothyroidism and celiac illness are more frequently observed. In addition to making diabetes management more complex, a concurrent autoimmune illness in type 1 diabetes patients can cause various clinical signs, ranging from slight complaints to potentially life-threatening circumstances in cases of adrenal inadequacy. Therefore, developing the best screening and treatment methods for people with T1DM is crucial. This will help them control their blood sugar levels and improve their quality of life (Bakker et al., 2013). Poor diabetes management and high TSH levels, which signify subclinical hypothyroidism and may impact diabetes control, may be linked to thyroid autoimmunity.

TNF-α is a pro-inflammatory cytokine part of the large cytokine family. It is primarily produced by macrophages and other innate immune system cells (Silva et al., 2019). TNF-α was first discovered as a factor contributing to tumour necrosis. Still, it has more recently been found to have other critical roles as a pathological factor in autoimmune disorders (Jang et al., 2021). According to our study's findings, TNF-α levels were significantly increased in T1DM patients compared to the healthy control group.

Similarly, TNF-α soluble receptor-2 (sTN-FRII) and plasma levels of IL-6 were elevated in diabetes patients compared with controls (Mohamed-Ali et al., 2001). The findings of this study are supported by the results of other researchers who found higher TNF- levels in children with T1DM, a tendency for higher TNF- gene expression, and high methylation in the TNF- gene promoter region. Also, another study concluded the possible role of TNF-α in the pathogenesis of type-2 diabetes mellitus (Swaroop et al.,2012; Arroyo-Jousse et al., 2016). Moreover, other studies documented that TNF -α is markedly elevated in the cytokine profiles of T1DM patients and strongly correlates with blood glucose levels and age (Jaleel et al., 2013, Seyfarth et al., 2017). Furthermore, in a meta-analysis study by Qiao et al. (2017), it was found that there was significant heterogeneity ($P < 0.001$) and that the serum TNF -α levels of T1DM patients were significantly higher than those of controls. In another study, Lechleitner et al. (2000) demonstrated that TNF- α concentrations were increased in T1DM and that these values were inversely correlated with HDL cholesterol amounts and positively correlated with HbA1c.

5. CONCLUSION

The results of this study pointed to a link between type I diabetes and thyroid illness. We suggested that patients with T1DM have higher rates of autoimmune thyroid levels than healthy controls with TSH elevation and high TNF- α levels. Moreover, thyroid TSH amounts were significantly increased in persons with T1DM, who were also associated with positive values of anti-TPO and anti-Tg. Thyroid autoimmune disease may be associated with poorly controlled diabetes and elevated TSH levels, which may indicate subclinical hypothyroidism and potentially affect diabetes management. A limitation of this work is the relatively few number of patients involved due to limited time and financial issues. As a result, we suggest that the present results be validated in a larger group of patients to confirm further the diagnostic and prognostic roles of detecting serum thyroid autoantibodies and TNF- α in T1DM patients. More research needs to be done to comprehend better how TNF-α contributes to the development of diabetes and its complications.

2. CONFLICT OF INTEREST

There is no conflict of interest in the current study.

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