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Original Research Paper

To Study the Levels of Cytokines in Hypothyroid Patients Suffering with Type 2 Diabetes Mellitus

Authors:
[1]Manikant Choudhary, [2]Dr. Shreya Nigoskar
[1]Ph. D scholar, Department of Biochemistry, Malwanchal university, Indore, MP
[2]Professor, Dept. Of biochemistry, Index medical college, Indore, MP
Corresponding Author:
Manikant Choudhary, Ph. D scholar, Department of Biochemistry, Malwanchal university, Indore, MP

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ABSTRACT:
Background: Hypothyroid patients affected with Type 2 diabetes mellitus (T2DM) are characterized by abnormalities in blood pressure, blood sugar, waist circumference, and cholesterol or triglyceride levels. Aim: To assess the effect of IL-1β, IL-2, IL-4, IL-5, IL-10, IL-13, IL-15, and TNF-α on health in hypothyroid patients affected with T2DM. **Materials & Methods**: The study included 100 hypothyroid patients affected with T2DM and 100 healthy controls. This research examined Indore Index Medical College & Research Centre patients. After approval, the study's researchers began. Before the study, each subject gave informed consent. Type 1 diabetes or fewer than five years of clinical symptoms and documented T2DM duration were excluded. Healthy controls were those without diabetes, multivitamins, or comorbidities. **Results**: The study examined the serum levels of IL-2, IL-4, and IL-15 in people with hypothyroid and T2DM and subjects in the control group and found significant statistical differences between the two groups. In contrast, the study found no statistically significant differences in IFN-γ and IL-15 levels across the groups. Conversely, healthy control individuals have exhibited increased serum levels of thyroxine, IL-2, and IL-15 compared to persons with hypothyroid and T2DM. Conclusion: Both pro-inflammatory and anti-inflammatory cytokines were shown to play a role in the progression of secondary diseases in hypothyroid patients with T2DM, as shown by the study.

Keywords: Hypothyroidism; Tumor necrosis factor; Cytokines; Body mass index; Inflammation.

INTRODUCTION:

Hypothyroid patients affected with Type 2 diabetes mellitus (T2DM) are characterized by issues with one's lipid profile (high or low cholesterol, high or low triglycerides). The cells of the immunity system produce cytokines, which modulate inflammation and responses to infection as cell-to-cell mediators [1-5]. According to studies, cytokines contribute to hypothyroidism in T2DM patients. Interleukin-6 (IL-6) and Tumour necrosis factor alpha (TNF-) are two inflammatory cytokines associated with these conditions. It is believed that these cytokines contribute to the development of inflammation in patients with hypothyroidism and T2DM [6-8]. Moreover, fat cells (adipose tissue) can produce inflammatory cytokines such as IL-6 and TNF-, which may promote systemic low-grade inflammation [7]. Because of this inflammation, insulin resistance may become more severe, and the risk of hypothyroidism in T2DM patients may increase. Inhibiting cytokines and reducing inflammation may therefore be a beneficial therapeutic strategy for hypothyroid patients with T2DM [8-10]. This category may include medications that target cytokines and inflammation, as well as lifestyle modifications such as exercising and consuming healthier. The successful identification of risk factors for such metabolic disorders [11-15] will facilitate the development of effective techniques for the early detection and prevention of disease. This is one of the goals that we want to achieve. The one-of-a-kind thing about this study is to assess the effect of IL-1β, IL-2, IL-4, IL-5, IL-10, IL-13, IL-15, and TNF-α on health in hypothyroid patients affected with T2DM.

MATERIALS AND METHODS:

The current study involved a total of 200 participants, with 100 individuals assigned to the group of hypothyroid patients affected by T2DM, and another 100 individuals assigned to the control group consisting of healthy persons. This study primarily focused on the individuals affiliated with the Index Medical College &

Research Centre located in Indore. The researchers commenced their work subsequent to receiving authorization from the relevant authorities. Prior to the commencement of the present study, informed consent was obtained from each participant representing the respective individual. The study included individuals who had been diagnosed with type 1 diabetes or had experienced pathological symptoms for less than five years and had a confirmed duration of type 2 diabetic mellitus (T2DM). The individuals classified as healthy controls exhibited the absence of diabetes, abstained from multivitamin usage, and did not manifest any comorbidities.

A certified physician from the medical department of the hospital conducted examinations on all participants in both groups. The physician adhered to normal protocols and took into consideration the exclusion and inclusion criteria of the study. The control group for health assessment comprised a sample of 100 individuals with matching age and gender characteristics, who were free from both hypothyroidism and T2DM A total of 100 patients diagnosed with hypothyroidism and comorbid T2DM were included in the second group of the study. T2DM was diagnosed utilising the diagnostic criteria published by the American Diabetes Association (ADA). The control group consisted of human volunteers of same age and sex who were in a normal glycemic condition. Every individual underwent a comprehensive evaluation conducted by a certified healthcare practitioner who adhered to established medical protocols. In both groups, each individual had a venous blood extraction procedure in which 5ml of fasting blood was collected into flat vials. This was accomplished using a sterile disposable syringe and needle in a controlled environment. After subjecting the samples to centrifugation at a speed of 3000 revolutions per minute (rpm) for a duration of 20 minutes, the resulting separation of serum from the blood was achieved. Subsequently, the samples were divided into smaller portions, known as aliquots, and stored at a temperature of 20°C.

The quantification of cytokines in the serum was performed using a multi-analyte Elisarray kit obtained from Qiagen laboratories. The affinity of capture antibodies towards their target protein is enhanced following the incubation process. Following the removal of unbound protein, the captured analyte can be specifically bound by detection antibodies that have been biotinylated and subsequently introduced into the designated wells. Following a final washing step, an avidin-horseradish peroxidase conjugate is employed to eliminate any residual unbound particles. Following a further washing step, a solution containing a colorimetric substrate is introduced into the wells. This substrate induces a blue coloration in the sample, the intensity of which is directly correlated to the concentration of the protein analyte present in the initial sample. By incorporating a stop solution, it becomes possible to detect the absorbance of the samples at a wavelength of 450 nm, enabling the ability to draw significant comparisons among them. A discrepancy of 4.9% was observed among the duplicates, while a discrepancy of 6.3% was observed among the tests. A sensitivity limit of 0.5 picograms per millilitre (pg/mL) was determined.

Statistical Analysis:

The statistical analysis was conducted using IBM SPSS version 20. The means of the variables in the two groups were compared using an unpaired "t" test. Additionally, calculations were performed to determine the percentages. A significance level of 0.05 was deemed to be statistically significant. In order to ascertain the association between two variables, regression analyses were employed. Additionally, calculations were performed to determine the percentages. A significance level of 0.05 was deemed to be statistically significant.

RESULTS:

Table 1 presents the elevated mean concentrations of IL-1, IL-5, IL-10, IL-13, and TNF- in both cohorts under investigation in the present study. Patients diagnosed with hypothyroidism who also had T2DM exhibited elevated levels of TNF-α and IL-1, in contrast to healthy individuals who demonstrated decreased levels of these cytokines. Furthermore, notable disparities in the levels of TNF-α and IL-1 in the serum were seen among the two cohorts. In contrast, healthy individuals exhibited elevated levels of weight, IL-2, and IL-15 in their blood when compared to individuals with hypothyroidism and comorbid T2DM. Significant disparities were seen in the aforementioned measures between patients diagnosed with hypothyroidism and T2DM and individuals without any known health conditions.

Table 1: Many cytokines were significantly reduced in the hypothyroid patients with T2DM compared to the controls.

Variable	Hypothyroid with T2DM Subjects (n=100)	Hypothyroid patients (n=100)	Healthy Controls (n=100)	P Value (ANOVA)
IFN-γ (pg/mL)	60.8 ± 9.7	62.3 ± 8.8	63.4 ± 9.5	>0.05
IL-2 (pg/mL)	49.6 ± 4.5	54.6 ± 9.2	58.7 ± 15.5	**<0.05**
IL-4 (pg/mL)	41.2 ± 11.9	47.8 ± 7.9	55.4 ± 15.5	**<0.05**
IL-12 (pg/mL)	158.9 ± 43.9	159.6 ± 66.8	160.4 ± 13.1	>0.05
IL-15 (pg/mL)	3.6 ± 1.2	5.1 ± 2.2	7.6 ± 2.8	**<0.05**

Cytokine levels were lower in T2DM patients with hypothyroidism, than hypothyroid patients than (see Table 2) compared to healthy controls. Blood levels of IL-2, IL-4, and IL-15 were significantly different between the hypothyroid patients with T2DM and the control group. However, no significant differences were identified when comparing IFN- and IL-15 levels between groups (Table 2).

Table 2: Many cytokines were significantly increased in the hypothyroid patients with T2DM, hypothyroid patients compared to the controls.

Variable	Hypothyroid with T2DM Subjects (n=100)	Hypothyroid patients (n=100)	Healthy Controls (n=100)	P Value (ANOVA)
IL-1β (pg/mL)	31.6 ± 5.0	25.7 ± 3.2	22.6 ± 0.6	**<0.0001**

IL-5 (pg/mL)	25.0 ± 2.1	22.5 ± 4.3	23.5 ± 0.6	>0.05
IL-10 (pg/mL)	25.2 ± 3.4	24.7 ± 5.6	24.1 ± 9.7	>0.05
IL-13 (pg/mL)	79.9 ± 42.9	76.6 ± 38.9	78.9 ± 10.8	>0.05
TNF-α (pg/mL)	8.3 ± 4.07	7.8 ± 5.4	5.7 ± 2.06	**<0.05**

DISCUSSION:

Patients with hypothyroidism and T2DM had significantly different blood concentrations of IL-4, IL-5, and IL-13 compared to the control group. It was shown that hypothyroid patients with T2DM had different blood sugar levels than those without the condition. This difference was seen in the serum concentrations of all three cytokines. Hypothyroid patients with T2DM were shown to have reduced serum IL-4 levels compared to healthy controls. Two separate groups were used for this analysis. This result was written up and published in a peer-reviewed scholarly journal. The available evidence at this time supports this claim [1]. According to the study results reported by him and his colleagues [2], IL-4 expression was significantly reduced in individuals with hypothyroid patients afflicted by T2DM. Patients with hypothyroidism and T2DM were shown to express the IL-4 gene at much higher levels than healthy controls [3]. The level of IL-4 was not linked to the existence of T2DM in hypothyroid individuals, according to another study [4]. Results from this study show that people with hypothyroidism and T2DM have lower levels of IL-4 production. When compared to healthy controls with typical IL-4 production, these findings are noteworthy.

Patients with hypothyroidism who developed T2DM showed greater levels of IL-5 and IL-13 than the healthy controls. Table 2 shows that these figures are far higher than was expected. Both IL-10 and IL-12 levels in the blood were similarly low in both groups. The ageing process raises the risk of this happening, even in otherwise healthy people. Increased levels of oxidised low-density lipoprotein (ox-LDL) have been linked to higher IL-5 levels in hypothyroid patients with T2DM [5]. Because there hasn't been a lot of recent research on IL5 and hypothyroid people with T2DM, we can only draw conclusions from older studies.

Serum levels of interleukin-2 (IL-2), interleukin-15 (IL-15), interferon- (IFN-), and interferon- were also compared between the two groups. Significant changes were found between the blood levels of TNF-, IL-2, IL-15, and IL-1 in hypothyroid patients with T2DM and those in healthy individuals. There were measurable shifts in the concentrations of IL-2, IL-15, and TNF-. The results of this study show that the levels of IL-2 rise steadily alongside body weight in healthy individuals. Furthermore, the association's effectiveness appears to be increasing over time. T-lymphocytes are controlled by IL-2, an anti-inflammatory cytokine [5-9]. Newly diagnosed hypothyroid patients with T2DM had significantly lower levels of IL-2, according to recent studies on the role of IL-2. Newly diagnosed hypothyroid patients with T2DM exhibited significantly lower levels of IL-2 compared to a control group of non-diabetic volunteers, according to recent research on the role of IL-2. Patients with hypothyroidism and T2DM exhibited higher IL-2 concentrations than the control group, according to the IL-2 research [10]. Diabetes Care [11-16] published the findings. These results were found to be markedly dissimilar to those of non-hypothyroid, T2DM-affected people. Patients with hypothyroidism who had T2DM for a duration of less than five years were the only ones considered for participation in this study. This is due to the fact that a person's ailment typically lasts for around five years. Our findings suggest that lower body mass index is a significant factor in the lower levels of IL-2 seen in hypothyroid patients with T2DM. This is a crucial element in lowering IL-2 concentrations. We found this by comparing people's height, weight, and IL-2 levels. In our study, the control group's normoglycemia may explain why they had higher weight and IL-2 levels than the hypothyroid patients afflicted by T2DM patients, as well as why there were statistically significant differences between the two groups. This is because we compared those with hypothyroidism and T2DM to people without either condition. This is due to the fact that studies have shown an inverse relationship between the prevalence of T2DM in hypothyroid patients and their body mass index. Our research led us to these conclusions. Hypothyroid patients with T2DM were found to have reduced amounts of anti-inflammatory cytokines [11,13].

In people with metabolic disorders, there was a negative correlation between IL-15 and blood weight, and a comparable correlation between TNF- and blood weight. This was the third piece of evidence we uncovered. Patients with hypothyroidism who develop T2DM are significantly thinner than healthy controls, and this, together with their increased production of IL-15 and TNF-, is enough to induce complications. This is due to the fact that hypothyroidism is associated with a higher risk of developing T2DM [16]. This correlation between hypothyroidism and T2DM has multiple possible explanations due to the multifaceted nature of the disease's aetiology. A recent study by Manohar M. found that elevated levels of the cytokine IL-15 are present in many tissues and organs. IL-15 has been found in the heart, liver, and kidneys, among others. This cytokine is crucial to comprehending the biology of the inflammatory response. As shown by [17], IL-15 is without a doubt involved in both the aetiology and development of inflammation in cardiovascular disease. Their investigation has led them to this conclusion. Multiple studies [11-14] found that atherosclerotic lesions in both humans and animals have abnormally high concentrations of IL-15. [11, 16] Diabetes and hyperglycemia are two additional risk factors for the development of atherosclerotic plaques.

High levels of TNF- have been associated to hypothyroidism, T2DM, obesity, and nephropathy in a number of studies [18–20]. The findings corroborate the observations [15,16] he made over the course of his inquiry. Two studies [18, 20] found that peripheral neuropathy patients' blood TNF- levels were considerably higher than those of the general population. Hypothyroid patients with T2DM also showed an association with the reported nerve conduction velocity. Our study's results match those of other studies in this area. Our noteworthy conclusion is that there is no significant association between weight and TNF- in the healthy control group. In the group of hypothyroid patients who also had T2DM, there was a strong correlation between the two factors. Treatment of secondary illnesses including cardiovascular and atherosclerotic lesions is profoundly affected by the pro-inflammatory cytokines TNF- and IL-15. Inflammation is a systemic effect of these cytokines. There is strong evidence that these cytokines play a role in the development of secondary problems. Our findings that hypothyroid patients with T2DM also had higher IL-15 and TNF- levels lend credence to this explanation. The finding gives this interpretation of the data more weight. Therefore, T2DM in hypothyroid patients is a common metabolic condition linked to a steady increase in inflammation, as seen by a rise in the levels of secreted cytokines including IL-15 and TNF- [18–20].

CONCLUSION:

Inflammatory cytokines IL-4, IL-5, and IL-13 were found to be associated with hypothyroidism in patients with T2DM. The findings are significant. New findings provide compelling evidence that both pro-inflammatory and anti-inflammatory cytokines contribute to the progression of secondary diseases in hypothyroid patients with T2DM. Furthermore, the studied cytokines may act as indicators for the early detection and diagnosis of secondary issues in hypothyroid patients with T2DM.

Conflict of interest: None.

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