
Evaluating Thyroid Hormones and Glycemic Parameters in Diabetic Patients: Insights from Kirkuk Governorate

Sarah Sami Hasan^{1*}, Israa Ghassan Zainal², Noorhan Ali Chelebi³

^{1*}Master student, College of Science, University of Kirkuk, Kirkuk, Iraq.

²Professor, College of Science, University of Kirkuk, Kirkuk, Iraq.

³Professor, College of Education for Pure Science, University of Kirkuk, Kirkuk, Iraq.

Email: ²israaz@uokirkuk.edu.iq, ³noorhanchelebi@hotmail.com

Corresponding Email: ^{1*}sccm22002@uokirkuk.edu.iq

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Abstract: *Chronic metabolic disorders like diabetes mellitus, which is typified by persistent hyperglycemia, can have a detrimental effect on a patient's quality of life and increase mortality rates. Complications include renal failure, systemic inflammation, and cardiovascular diseases are common. Because poor protein synthesis and glucose metabolism increase the risk of subsequent difficulties, co-occurring hyperthyroidism in diabetes individuals exacerbates glucose management even more. Plasma protein levels can be dramatically affected by the dysregulation of proteins, such as albumin and globulin, which are essential for many physiological processes and can be dysregulated in diseases including diabetes and hyperthyroidism. By comparing the levels of total protein, albumin, globulin, and oxidative stress biomarkers, such as free amines, thiols, and carbonyls, in diabetic patients with and without hyperthyroidism to healthy controls, this study investigates the molecular pathways causing diabetes complications. Significant differences in oxidative stress and protein indicators are found in these patients, which sheds light on the biochemical abnormalities they have and opens up new therapy options that target oxidative stress and protein metabolism.*

Keywords: *Diabetes Mellitus, Hyperthyroidism, Oxidative Stress, Protein Metabolism, Glycation, Reactive Oxygen Species (ROS).*

1. INTRODUCTION

Chronic metabolic disease known as diabetes mellitus is mostly characterized by persistent hyperglycemia, which can be caused by insulin resistance or insufficiency [1]. This illness can cause a number of consequences, including systemic inflammation, renal failure, and



cardiovascular illnesses, which can have a major negative impact on quality of life and raise mortality rates [2]. Chronic hyperglycemia in diabetes can cause problems, mostly from inflammation and metabolic disruptions that impact the kidneys, heart, nerves, eyes, and blood vessels [3]. Renal insufficiency, neuropathy, and cardiovascular disorders are common consequences. Diabetes management is even more difficult when hyperthyroidism coexists because thyroid hormones further impair protein synthesis and glucose metabolism [4]. This combination makes diabetes patients' glucose control worse and increases their likelihood of developing new problems [5]. Proteins are vital macromolecules that carry out a wide range of tasks in the human body, including antioxidant activity, metabolite transportation, and osmotic pressure maintenance. Because they are involved in so many physiological processes, albumin and globulin stand out among these proteins [6].

The regulation of protein metabolism can be upset in diseases including diabetes and hyperthyroidism, which can result in significant changes in plasma protein levels. This dysregulation has serious consequences for patient health and contributes to conditions like nephropathy and cardiovascular disease [7]. It is not only a biochemical aberration. The albumin/globulin (A/G) ratio is a useful biomarker for evaluating systemic inflammation and liver function. It also helps identify underlying inflammatory processes that could aggravate pre-existing diseases and provides information about patients' overall metabolic status. Gaining insight into these connections is essential to enhancing patient outcomes for people with long-term metabolic diseases [8].

It is becoming more widely acknowledged that oxidative stress plays a major role in the etiology of a number of metabolic diseases, such as diabetes and hyperthyroidism. This disorder is caused by an imbalance between the generation of reactive oxygen species (ROS) and antioxidant defenses, which damages and malfunctions cells. When diabetes is present, oxidative stress plays a role in the build-up of advanced glycation end products (AGEs), which worsen insulin resistance and other metabolic problems [9]. Research has demonstrated that increased plasma concentrations of thiols, free amines, and carbonyl compounds are indicators for oxidative damage, indicating weakened antioxidant defenses in afflicted individuals [10]. These metabolic illnesses and oxidative stress interact, underscoring the need for a more thorough comprehension of their underlying mechanisms. For example, increased ROS generation linked to hyperthyroidism may exacerbate insulin sensitivity and play a role in the development of thyroid diabetes [11].

Therefore, investigating the connection between hyperthyroidism, diabetes, and oxidative stress is essential for creating treatment approaches that effectively reduce oxidative damage and enhance patient outcomes [12]. This study intends to examine the levels of total protein, albumin, globulin, free amines, thiols, and carbonyls in the plasma of diabetic patients, with and without hyperthyroidism, and compare them with healthy individuals, given the growing interest in understanding the molecular mechanisms underlying diabetes complications. The goal of the study is to determine the degree of oxidative stress and protein malfunction in these conditions by looking at these indicators. This information may help develop future treatment plans for diabetes and its consequences.



2. RELATED WORKS

[13] concluded that there was no significant difference in carbonyl levels; however, significant differences were observed in total protein, albumin, globulin, albumin/globulin ratio (AGR), free amino acids, free amino/total protein ratio, thiol levels, and thiol/total protein ratio, as well as carbonyl/total protein levels across all studied groups. Correlation analysis revealed significant positive relationships between total protein and globulin, AGR and albumin, and free amino acids and albumin. Conversely, significant negative correlations were found between total protein and AGR, as well as AGR and globulin in the context of diabetic complications. The findings suggest that oxidation markers may be relevant for monitoring diabetic complications. [14] The objective of their study was to ascertain how people without diabetes who have overt hyperthyroidism was affected by their thyroid condition and if these alterations in HbA1c are reversed once they reach euthyroidity. Without a change in glucose levels. These levels sharply dropped when they achieved euthyroidism. Patients with hyperthyroidism did not show any significant changes at baseline or after therapy. [15] investigated how natural compounds can prevent protein glycation and came to the conclusion It has been discovered that several natural substances, particularly polyphenols, effectively prevent protein glycation in vitro. Their bioavailability issues make their activity in vivo more challenging. However, there are certain advantages to using natural antioxidants to counteract the effects of excessive glycation. There is reason to believe that natural substances used as food additives may avoid the negative consequences of protein glycation and, thus, postpone aging, even if their mechanisms of action may extend beyond direct prevention of glycation. Limiting the amount of AGE in the meal may be another helpful strategy.

Thiols are known to be one of the primary protective mechanisms of the body against oxidative stress. They have been shown to play important roles in enzymatic reactions, programmed cell death, detoxification, and antioxidant protection within the body. Numerous studies have demonstrated alterations in thiol status and the thiol/disulfide balance in various diseases, including gastrointestinal, respiratory, reproductive, urinary, metabolic disorders, and cancer. This also indicates that thiol status is critical in the etiology of diseases resulting from oxidative stress [39]. The carbonyl results are consistent with the findings of Bora and Adole [40], who studied cardiovascular diseases and the Fenton reaction in vitro on human albumin, confirming the antioxidant activity in patients with human albumin. Disruption in the concentration of carbonyls and carbonyl/human albumin-like proteins may result from oxidative stress and inflammation in patients.

3. METHODOLOGY

Patient Sample Collection and Study Duration

This study included collecting blood samples from 120 diabetes mellitus patients, including 40 with hyperthyroidism, 40 with hypothyroidism, and 40 without thyroid issues, aged 20-70, from various hospitals in Kirkuk. Additionally, 50 samples from healthy individuals of the

same age range were included. The research was conducted in Kirkuk between November 2023 and April 2024.

Blood Sample Collection

Five milliliters of venous blood were collected from each participant after consent. Two milliliters were placed in an EDTA tube for glycated hemoglobin analysis via immunofluorescence. The rest was used for serum extraction, centrifuged at 3000 rpm for 10 minutes. The serum was stored at -20°C for measuring triiodothyronine, thyroxine, thyroid-stimulating hormone, liver enzymes, and viral hepatitis markers.

Estimation of Total Protein, Albumin, Free Amino Groups, Thiol Groups, and Carbonyl Groups in all Studied Groups

The total protein level in plasma samples was determined using the Lowry method [16], with bovine serum albumin (BSA) as the standard. The albumin level was determined using the Bromo Cresol Green method [17]. The spectrophotometric estimation of free amino groups was performed according to the method of [18]. The estimation of thiol groups was performed according to Ellman's method [19]. The carbonyl group in the protein was estimated using the method by Levine et al.[20].

Purification of Albumin

Albumin was purified from plasma using ammonium sulfate precipitation according to a modified procedure by Jovanović [21].

Estimation of Glycation Levels in Crude and Partially Purified Human Serum Albumin (HAS)

Glycation levels were measured using the thiobarbituric acid (TBA) method as described by [22].

Figures (1, 2, and 3) show the standard curves for the estimation of total protein, free amino groups, and glycation, respectively.

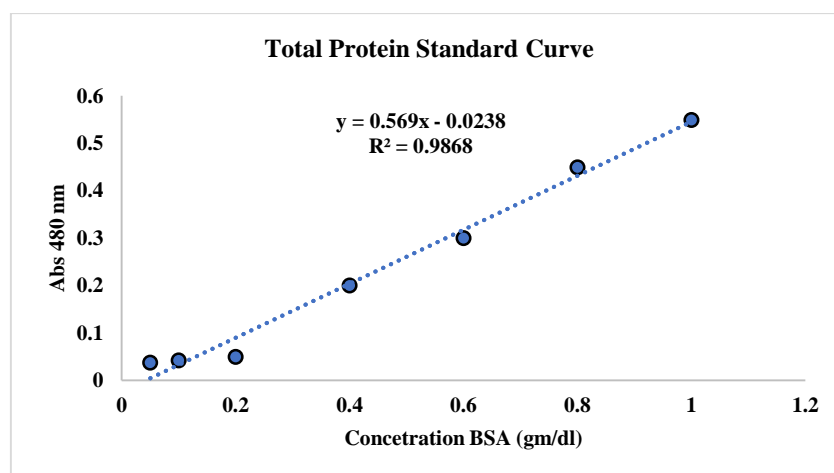


Fig. 1. Standard curve of total protein estimation.

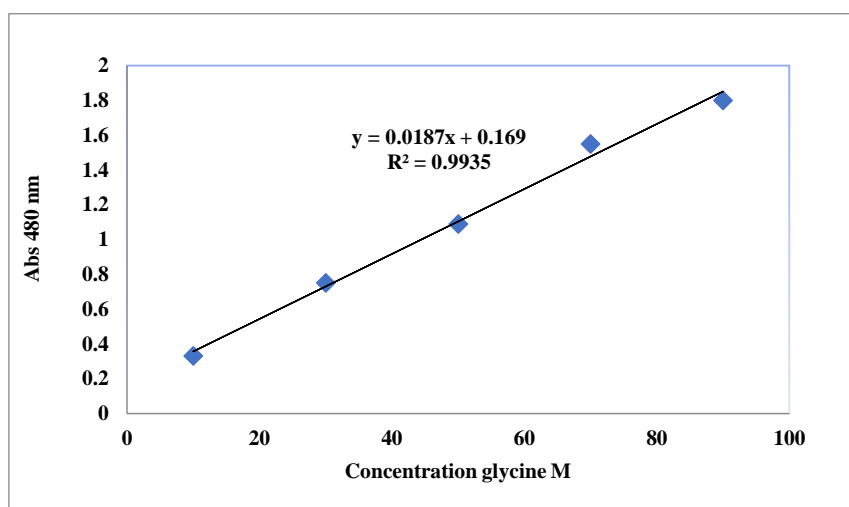


Fig. 2. Standard curve of free amine concentration.

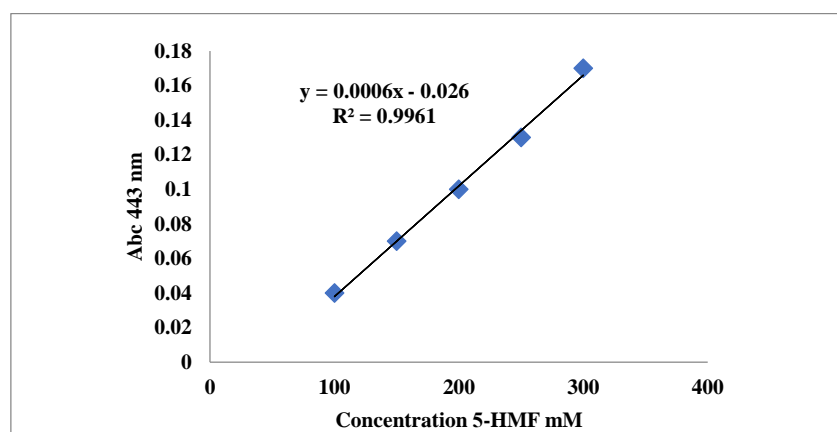


Fig. 3. Standard curve for the estimation of glycation concentration.

4. RESULTS AND DISCUSSIONS

Estimation of Total Protein (TP), Albumin, Globulin Levels, and Albumin/Globulin (A/G) Ratio in all Studied Groups

Table 1. presents the levels of total protein (TP), albumin, globulin, and the albumin/globulin ratio, expressed as (Mean \pm Standard Deviation SD) (g/100 ml), in the plasma of the studied groups. The patient group was divided into a diabetes group and a group with both diabetes and hyperthyroidism, compared to healthy individuals. The results showed a significant increase in total protein concentration in the diabetes group (9.39 ± 0.468) compared to the diabetes with hyperthyroidism group (4.90 ± 0.12) and the healthy group (6.60 ± 0.29). Additionally, a significant increase in albumin concentration was observed in the diabetes group (6.126 ± 0.312) compared to the diabetes with hyperthyroidism group (1.240 ± 0.41) and the healthy group (4.90 ± 0.127). Furthermore, there was a significant increase in globulin concentration in the diabetes with hyperthyroidism group (3.66 ± 0.39) compared to the diabetes group (3.294 ± 0.36) and the healthy group (2.53 ± 0.34). The results also



revealed a significant increase in the albumin/globulin ratio in the diabetes group (1.87 ± 0.18) compared to the diabetes with hyperthyroidism group (0.3585 ± 0.21) and the healthy group (1.632 ± 0.21).

Table 1. Total protein, albumin, globulin, and albumin/globulin ratio in the plasma of diabetic patients, diabetic patients with hyperthyroidism, and healthy individuals.

Parameters	Control (Mean \pm SD)	Diabetes (Mean \pm SD)	Diabetes & Hyperthyroidism (Mean \pm SD)
TP(gm/dl)	6.606 ± 0.2921	9.397 ± 0.4682^a	$4.903 \pm 0.1274^{a,b}$
Albumin(gm/dl)	4.903 ± 0.1274	6.126 ± 0.3120^a	$1.240 \pm 0.4140^{a,b}$
Globulin(gm/dl)	2.534 ± 0.3444	3.294 ± 0.3686^a	$3.663 \pm 0.3957^{a,b}$
Albumin/Globulin ratio	1.632 ± 0.2165	1.877 ± 0.1876^a	$0.3585 \pm 0.2173^{a,b}$

Proteins exist as enzymes, hormones, and antibodies, and they also maintain osmotic pressure balance in the blood. Albumin is one of the most abundant components in human plasma [23]. The results of this study may be attributed to elevated glucose levels in patients, as the normal filtration process in the kidneys is altered, leading to the accumulation of toxic waste and significant losses of total protein, albumin, and globulin in the urine. This is why albumin and globulin levels are low in patients with diabetes complications and hyperthyroidism [24]. Albumin and globulin function as transport proteins and biomarkers in the context of diabetes complications [25]. Albumin constitutes over 50% of plasma proteins and is synthesized by the liver. Its physiological functions include regulating osmotic pressure, transporting nutrients, fatty acids, bilirubin, cholesterol, and drugs in the bloodstream, and waste removal [23]. Studies have shown that albumin plays a crucial role in the antioxidant capacity of blood plasma against free radicals [26]. The albumin-to-globulin ratio (AGR) was also measured, which can provide insights into bodily issues [27]. This representative parameter can be easily measured and at a low cost. Generally, albumin and globulin are primarily produced by liver cells [28]. The results regarding total protein, albumin, globulin levels, and the albumin-to-globulin ratio were consistent with findings by Abd [29], who studied these parameters in cases of beta-thalassemia. Additionally, the total protein results were also consistent with those of Azeez [30], who examined these parameters in breast cancer patients and diabetic nephropathy, respectively. Individuals with hyperthyroid diabetes have lower albumin levels. The most straightforward explanation for this is inflammation, which is associated with decreased albumin concentration and increased risk of cardiovascular diseases. Several hypotheses have been proposed to explain the mechanisms that may help clarify the relationship between low serum albumin levels and heightened risk of cardiovascular diseases. When systemic inflammation occurs, the liver produces serum albumin, which is considered a negative acute-phase protein. However, the liver also produces other acute-phase proteins in addition to human albumin [31]. Another mechanism is that a decrease in plasma albumin concentration, which cannot be detected and captured by C-reactive protein (CRP), reflects increased vascular permeability during inflammatory processes. Fluid redistribution to the interstitial space can lead to significant and rapid changes in plasma albumin concentration. Furthermore, multiple factors influence the



reduction of albumin concentration in individuals who also suffer from diabetes and cardiovascular diseases. First, diabetes may result in decreased hepatic albumin production [32]. Second, processes related to glycated albumin formation can lead to lower albumin levels. Glycated albumin acts as a precursor for advanced glycation end products (AGEs), which induce oxidative stress and inflammation and have an aberrant ability to bind to various ligands. Additionally, there is evidence that glycated albumin (and other glycosylated proteins) elicits an immune response that results in reduced albumin concentration, and increased oxidative stress has been linked to the development of insulin resistance and diabetes. Third, as previously mentioned, inflammation plays a role in the progression of diabetes. Finally, hazardous waste accumulates due to excessive glucose concentration and the natural filtration process of the kidneys. Furthermore, urine loses a significant amount of albumin [33].

Estimation of Free Amine and Free Amine/TP Levels in the Plasma of all Studied Groups

The results in Table 2. represent free amine and free amine/TP levels expressed as (mean ± standard deviation) in units of (mmol/L) in the plasma of the studied groups. The results showed a significant increase in free amine concentration in the diabetic group, reaching (55.29 ± 2.35) compared to the diabetic and hyperthyroid group (48.48 ± 1.45) and the healthy group (32.24 ± 1.18). The results also indicated a significant increase in free amine/TP concentration in the diabetic group, reaching (9.891 ± 0.34) compared to the diabetic and hyperthyroid group (5.89 ± 0.36) and the healthy group (4.887 ± 0.29).

Table 2. Levels of free amine and free amine/TP in the plasma of all studied groups.

Parameters	Control (Mean ± SD)	Diabetes (Mean ± SD)	Diabetes & Hyperthyroidism (Mean ± SD)
Free amino (mmole/L)	32.24 ± 1.184	55.29 ± 2.359 ^a	48.48 ± 1.450 ^{a,b}
Free amino/TP (mmole/gm)	4.887 ± 0.2980	5.893 ± 0.3660 ^a	9.891 ± 0.3472 ^{a,b}

The results indicated a significant increase ($p \leq 0.05$) in the levels of amines and free amine/TP in diabetic patients. These findings were consistent with those reported by Li et al. [34] and the study by Noah [35], where they also found increases in the concentrations of free amino acids and free amino acids/total protein in patients suffering from diabetes and cardiovascular diseases, respectively. The rise in amines and free amines/TP may be a result of increased concentrations of branched-chain amino acids (BCAAs) and central nervous system (Acyl CNsO) associated with fatty acids in diabetic patients [36]. Branched-chain amino acid (BCAA) levels increase in cases of fasting and diabetes; however, the etiology of the disease has not been elucidated. It has been shown that BCAA metabolism primarily occurs in muscles due to the high activity of BCAA aminotransferase, which converts BCAAs and α -ketoglutarate (α -KG) into branched-chain keto acids (BCKAs) and glutamate. The loss of α -KG from the acid cycle (metabolism) is mitigated by the conversion of glutamate back to α -KG in the alanine aminotransferase and aspartate aminotransferase

reactions, where glycolysis serves as the main source for the amino group acceptors, pyruvate and oxaloacetate. The irreversible oxidation of BCKA by dehydrogenase is sensitive to BCKA supply and the ratios of NADH to NAD⁺ and acyl-CoA to CoA-SH. It is suggested that reduced glycolysis and increased fatty acid oxidation, characteristics of fasting and diabetes, cause changes in muscle leading to elevated BCAA levels. The main changes include (a) impaired BCAA transport due to reduced supply of amino group acceptors (α -KG, pyruvate, and oxaloacetate) and (b) the inhibitory effect of NADH and acyl-CoAs produced in fatty acid oxidation on the citric acid cycle [37].

Estimation of Thiol, Thiol/TP, Carbonyl, and Carbonyl/TP Levels in the Plasma of Studied Groups

The results in Table 3. show the levels of thiol and thiol/TP expressed as (mean \pm standard deviation) in units of (nanomole/mL) in the plasma of the studied groups. The results demonstrated a significant increase in thiol concentration in the diabetic group, which was (36.91 ± 0.463) compared to the diabetic and hyperthyroid group (35.52 ± 0.45) and the healthy group (22.01 ± 0.65). The results also indicated a significant increase in the concentration of thiol/TP in the diabetic and hyperthyroid group, which was (7.251 ± 0.17) compared to the diabetic group (3.934 ± 0.19) and the healthy group (3.329 ± 0.14). Additionally, the results in Tables (3) showed the levels of carbonyl and carbonyl/TP expressed as (mean \pm standard deviation) in units of (mmol/L) in the plasma of the studied groups. The results demonstrated a significant decrease in carbonyl concentration in the diabetic and hyperthyroid group, which was (76.84 ± 1.13) compared to the diabetic group (78.18 ± 1.40) and the healthy group (82.59 ± 1.85). The results indicated a significant increase in the concentration of carbonyl/TP in the diabetic and hyperthyroid group, which was (15.81 ± 0.74) compared to the diabetic group (8.337 ± 0.45) and the healthy group (12.52 ± 0.51).

Table 3. Levels of thiol and thiol/total protein, carbonyl and carbonyl/ total protein in all studied groups.

Parameters	Control (Mean \pm SD)	Diabetes (Mean \pm SD)	Diabetes & Hyperthyroidism (Mean \pm SD)
Thiol(nmole/ml)	22.01 ± 0.6585	36.91 ± 0.4638^a	$35.52 \pm 0.4556^{a,b}$
Thiol/TP(nmole/gm)	3.329 ± 0.1463	3.934 ± 0.1900^a	$7.251 \pm 0.1753^{a,b}$
Carbonyl(mmole/L)	82.59 ± 1.856	78.18 ± 1.404^a	$76.84 \pm 1.130^{a,b}$
Carbonyl/TP(mmole/gm)	12.52 ± 0.5105	8.337 ± 0.4576^a	$15.81 \pm 0.7466^{a,b}$

The thiol results align with the study by Pullalah et al. [38]. The elevated thiol levels in diabetic patients can be attributed to the protective role of antioxidants in these conditions, as this group of patients is at significant risk of tissue oxidation due to compromised plasma antioxidant defenses. Among all antioxidants present in the body, thiols constitute the largest portion of the total antioxidants and play a crucial role in defending against reactive oxygen species. Oxidative stress effects arise from oxidative damage to macromolecules and cell membranes, alongside the disruption of metabolic activities in cellular components within



living organisms. It is well established that organ and tissue diseases occur when oxidative stress is excessive in the body. Persistent hyperglycemia and inhibition of metabolic enzymes and detoxification lead to excessive synthesis of carbonyl compounds, resulting in carbonyl stress [41].

Purification of Plasma Albumin from Patients with Diabetes, Hyperglycemia, and Hyperthyroidism

Albumin was purified using the ammonium sulfate precipitation method, and the results have been summarized in Table 4. below:

Table 4. Methods for purifying albumin in the plasma of diabetic patients, hyperglycemia, and hyperthyroidism.

Samples	Volum (ml)	Albuman in diabetes	Yield%
Crude serum	5ml	6.375	100%
Pellet (AS) 85%	5ml	5.519	86%
Samples	Volum (ml)	Albumin in diabetes& thyperthyroidism	Yield%
Crude serum	5ml	1.519	100%
Pellet (AS) 85%	5ml	1.061	93%

The human albumin serum indicated through ammonium sulfate precipitation that the precipitate obtained from diabetic patients suffering from hyperthyroidism and diabetes was (93%) and (86%), respectively, compared to the controls after precipitation, which was (85%). Levels of glycation in human serum albumin after incubation with vitamins C and B2 are shown in Table 5 .

Table 5. shows the levels of glycation in human serum albumin after incubation with vitamins C and B2.

Groups	Glycation Conc. mmol/mg protein (Mean ± SD)
HSA- AGE in patients before inhibition	258.3
Vitamin C (0.00001)	113.3
Vitamin C (0.00005)	86.5
Vitamin C (0.0001)	63.7
Vitamin B2 (0.00001)	123.3
Vitamin B2 (0.00005)	96.5
Vitamin B2 (0.0001)	71.6

The level of glycation decreases with increasing levels of ascorbic acid over time. This study confirmed that ascorbic acid affects protein glycation. Ascorbic acid is a compound with two ionizable hydroxyl groups (pKa₁=11.8, pKa₂=4.25), which appear as ascorbate anions at physiological pH. The hydroxyl group present in ascorbic acid exhibits a strong interaction with the amino group of proteins [42]. The mechanism by which ascorbic acid inhibits protein glycation is highly complex [43]. Numerous previous observations suggest that



ascorbic acid can compete with glucose for binding to proteins and inhibit their glycation [44]. The results indicated that ascorbic acid inhibits protein coagulation under different conditions when present at <80% (184), and Davie et al. [45] found that ascorbic acid can also inhibit hemoglobin coagulation. Furthermore, Kim et al. [46] reported that glycosylated albumin produced by antioxidants such as ascorbic acid reduces cellular toxicity and cell death in cultured bovine retinal pericytes. The levels of ascorbic acid used in this study are consistent, and it interacts with glycoproteins, positively impacting the reduction of chronic levels in plasma [47]. It was found that vitamin C is a stronger inhibitor than vitamin B2.

5. CONCLUSIONS

The study found substantial differences in total protein, albumin, globulin levels, and albumin/globulin ratios in diabetic individuals, particularly those with concurrent hyperthyroidism. The findings show that the observed protein imbalances are linked to kidney dysfunction, inflammation, and oxidative stress, all of which are associated with diabetes and cardiovascular disease. Increased levels of branched-chain amino acids (BCAAs), free amines, and thiols in diabetic patients emphasize the role of oxidative stress and inflammation in disease progression. Additionally, albumin glycation is influenced by antioxidant vitamins, particularly ascorbic acid, which plays a protective function against protein degradation. These findings highlight the need of tracking protein levels and oxidative indicators in order to better understand disease causes and the effectiveness of therapeutic approaches in diabetes and hyperthyroidism.

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