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Assessment of Some Biochemical Markers (Protein Levels, Hepatic Enzymes, Calcium, and Iron) Status in Patients of Diabetic Nephropathy in Thi-Qar/ Iraq

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Abstract: **Diabetic nephropathy is a kidney disease that develops as a complication of diabetes, and it is issociated with renal impairment with increasing age. Renal impairment among diabetic individuals can be detected using serum markers of glomerular filtration rate and microalbuminuria. This study was carried out at AL-Hussein Hospital and Consulting Laboratories .The study period extended from October 2024 to January 2025. aiming to assess the biochemical changes in kidney failure patients with and without diabetes. Based on the results, it was noted that diabetic patients had higher levels of total protein and lower levels of albumin compared to the healthy control group. Additionally, patients with diabetic nephropathy had elevated levels of liver enzymes (ALT, AST, and ALP), C-reactive protein (CRP) ,and calcium. A noticeable lower iron levels were observed in both diabetic and diabetic nephropathy groups compared to the healthy control group.**

***Keywords*: Diabetic nephropathy, Dialysis, Total protein, Liver enzymes, Renal failure.**

I. INTRODUCTION

Diabetic nephropathy (DN) is a serious complication of both type 1 and type 2 diabetes [1]. It impairs the kidneys' normal function of removing waste and excess fluid from the body. Over the years, DN gradually damages the kidneys' filtration system. Early treatment can prevent or slow the condition and reduce the risk of complications [2]. It is responsible for approximately 40% of cases of end-stage renal disease (ESRD) [3]. The prevalence of diabetic mellitus is steadily increasing worldwide. The World Health Organization's Global Diabetes Report, released on April 6, 2016, pointed out that the prevalence of diabetes in the global adult population is 8.5% [4]. Abusaib *et al*. found that about 1.4 million Iraqis suffer from diabetes. The main cause of renal failure in Iraq is diabetes (33%), followed by hypertension (22.6%). The prevalence of diabetic nephropathy among Iraqi patients with type 2 diabetes is 16.1% [5]. Assessment of biochemical indicators is important indicators in the lives of patients with kidney disease or diseases that affect kidney function [6]. proteins are found in all body fluids but show a very high concentration in plasma, lymphatic fluids and some secretions, the total serum protein test evaluates the total amount of proteins in the blood, as many studies have shown high levels of globulin in the blood and a decrease in the ratio of albumin to globulin (A/G) in patients with infections and advanced malignant tumors [7].

Alkaline phosphatase (ALP), aspartate transaminase (AST), and alanine transaminase (ALT) are routine indexes of liver function. The liver plays an important role in glucose homeostasis. Evidence of deranged liver functions is observed in long-standing DM , and a slight elevation of liver enzymes is seen in insulin resistance [8]. Insulin resistance results in the production of free fatty acids ,which damage the liver parenchyma and result in the elevation of the liver enzymes, especially AST, ALT, and ALP could serve as a strong predictor for DN[9].C-reactive protein (CRP) is an Acute- phase protein that is part of the pentraxin protein family. It is primarily produced by liver cells and in small quantities by some other cells, like vascular smooth muscle cells and macrophages. An elevated CRP level is considered a biomarker for inflammatory response, tissue injury, and chronic progression of diseases [10] . Diabetes is linked with a change of homeostasis for the plasma iron in humans, that have the ability to produce reactive oxygen species (ROS) induced by this disease and its microangiopathy complication especially diabetic nephropathy (DN) [11].

II. MATERIALS AND METHOD

A. Sample Collection and Preparation of Blood Serum

This study included 160 samples of(healthy, diabetes mellitus, nephropathy, diabetic nephropathy) patients aged more than 35years who were present in the dialysis unit of Imam Hussein Hospital and Consulting Laboratories. The study period was from October, 2024 to January, 2025. About 3 mL of blood was placed in a microcentrifuge tube, then let the blood clot by leaving it undisturbed at room temperature for 15–30 minutes; Afterward, the clot was removed by centrifuging at 1000–2000 rpm/min, for 10 minutes in a refrigerated centrifuge.

B. Biochemical Tests

The concentrations of a set of biochemical markers in the studied groups, including total protein, albumin, liver enzymes, iron, and calcium, were determined using ready-to- use diagnostic kits from the china company BT Lab [12]. These analyses were performed using the Neo Chem 20

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semi-automated biochemical analyzer. The method is based on colorimetric reactions, and the absorbance of each sample was measured using the integrated spectrophotometer [13].

C. *Determination of C-Reactive Protein (CRP)*

The Assay Procedure: One drop of serum was placed on the slide using a disposable plastic dropper in a circular area of the slide. One drop of CRP Latex reagent was then added to the drop above and mixed with a disposable applicator stick. The slide was gently shaken back and forth, and agglutination was observed visually after 2 minutes [14].

D. *Statistical Analysis*

The statistical analysis of the results was performed using the SPSS software program based on the T-test, where the averages of patients and healthy were compared at a significant level ($P \leqslant 0.05$). The ANOVA test was used to compare the age groups at a significant level ($P \leqslant 0.05$), and the values of the variables were described as a standard deviation $\pm$ Mean [15].

III. RESULTS

A. *Total Protein*

In the table shown (1), the highest value was recorded in the age group (35-50) in diabetic nephropathy patients (8.28±0.97), while the lowest value was in healthy individuals (0.15±4.86). In the age group (51-65), it was also noted that there was an increase in the levels of Total protein in diabetic nephropathy patients (9.88±0.91) compared with patients of nephropathy and diabetes, similar values were shown (6.89±0.44 and 6.21±0.5, respectively). The lowest value was in healthy individuals (0.29±4.55). In patients over 66 years of age, the highest value was also shown in diabetic and diabetic nephropathy patients (8.85±0.92) compared to healthy individuals. In addition, the overall means of the total sample confirm that total protein levels are markedly higher in diabetic and diabetic nephropathy patients compared to healthy individuals, which reflect the impact of nephropathy on kidney function.

Table 1: Serum total protein levels in the control group, diabetes mellitus, nephropathy, and diabetic nephropathy patients across different age categories

Total protein													LSD
Group	Control			DM			N			DN			
	N	Mean	Std. Deviation	N	Mean	Std. Deviation	N	Mean	Std. Deviation	N	Mean	Std. Deviation	
35-50	9	4.86 aC	±0.15	10	4.62 cD	±0.12	8	7.31 aB	±0.37	11	8.28 cA	±0.97	0.214
51- 65	16	4.55 aD	±0.29	14	6.21 bC	±0.5	13	6.89 bB	±0.44	12	9.88 aA	±0.91	0.115
66 <	15	5.13 aD	±0.22	16	6.59 aC	±0.6	19	7.03 bB	±0.30	17	8.85 bA	±0.92	0.102
LSD		1.563			0.213			0.225			0.174		
Total	40	5.08	1.06	40	5.48	0.60	40	6.34	0.67	40	9.17	4.13	2.835

- Values expressed as Mean ±SD , DM= Diabetes Mellitus , N = Nephropathy , DN= Diabetic Nephropathy
- Different capital letters denote significant differences (P≤0.05) between groups. Different lowercase letters indicate significant differences (P≤0.05) between age groups of the same group.

B. *Albumin Level*

The results in Table (2) showed a significant difference (P 0.05). In the age group (35-50), albumin levels were lower in diabetic patients (3.600 ± 0.385), while the values in the nephropathy group and the healthy group were similar (3.737 ± 0.440 and 3.733 ± 0.484, respectively). As for patients older than 51-65, albumin levels remained lower in diabetic patients compared to the other groups. As for individuals aged 66 years, increased albumin levels were observed in nephropathy patients and decreased albumin in diabetic patients. In addition, the general averages of the total sample confirm that albumin levels were significantly lower in diabetic patients compared to the other groups and the healthy groups, reflecting the impact of diabetes on kidney function.

Table 2: Serum albumin levels in the control group, diabetes mellitus, nephropathy, and diabetic nephropathy patients across different age categories

Album													LSD
Group	Control			DM			N			DN			
	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	
35-50	9	3.733 bB	0.484	10	3.600 bB	0.385	8	3.737 bB	0.440	11	5.376 aA	2.208	1.64303
51- 65	16	3.912 bAB	0.741	14	3.614 bB	0.409	13	3.707 bB	0.619	12	4.766 aA	2.110	1.05897
66 <	15	4.166 aA	1.527	16	3.700 aB	0.382	19	4.142 aA	1.392	17	4.135 aA	1.987	0.46667
LSD		0.4333			0.100			0.43441			1.24107		
Total	40	3.96	1.061	40	3.64	0.385	40	3.92	1.045	40	4.66	2.096	1.3960

C. ALT Level

Table (3) showed a significant difference (P≤0.05). In the age group 35-50, diabetic nephropathy patients showed the highest ALT levels (27.71) compared with the group of nephropathy, diabetes, and healthy patients (18.47±10.51, 16.38±7.88, and 11.64±5.53 , respectively). While the ALT level in diabetic nephropathy patients decreased patients in over the age of 51-65, or 66 < . In addition, the overall mean for the total sample confirm that ALT levels are markedly higher in diabetic nephropathy patients compared with the healthy group and other groups, which reflecting the effect of diabetic nephropathy on kidney function.

Table 3: Serum ALT levels in the control group, diabetes mellitus, nephropathy, and diabetic nephropathy patients across different age categories.

ALT													
Group	Control			DM			N			DN			LSD
	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	
35-50	9	11.64 aB	5.53062	10	16.38 aB	7.88103	8	18.47 aAB	10.51146	11	27.71 aA	11.36634	9.423
51- 65	16	11.88 aB	4.34985	14	16.94 aAB	8.51684	13	17.24 aAB	10.19899	12	20.16 cA	13.32007	8.279
66 <	15	10.85 aB	3.50324	16	14.16 bB	6.24456	19	12.98 aB	6.56987	17	23.29 bA	10.31846	9.131
LSD		1.04			2.79			5.485			3.127		
Total	40	11.44	4.26398	40	15.69	7.42649	40	15.47	8.81253	40	23.57	11.63185	4.025

D. AST Level

In the current study, Table (4) showed significant differences (p≤0.05). The highest AST levels were found in patients with nephropathy, over the age of (35-50) compared to the diabetic nephropathy group, and the diabetes, and healthy group. The lowest group was the healthy group. For the age group (51-65), AST levels decreased in patients with nephropathy, and the diabetic and healthy groups showed similar values (16.285±5.35549 and 16.500±5.2662). There was also an increase in patients with diabetic nephropathy and diabetes for patients over the age of 66< years compared to other age groups. In addition, the overall means for the total sample confirm that AST levels are markedly higher in nephropathy patients, compared to other groups, and the healthy group, which reflects the impact of nephropathy on kidney function.

Table 4: Serum AST levels in the control group, diabetes mellitus, nephropathy, and diabetic nephropathy patients across different age categories.

AST													
Group	Control			DM			N			DN			LSD
	N	Mean	Std. Deviation	N	Mean	Std. Deviation	N	Mean	Std. Deviation	N	Mean	Std. Deviation	
35-50	9	14.777bC	6.6478	10	3.000 aB	8.64099	8	46.875aA	8.52622	11	16.454cC	3.58786	11.222
51- 65	16	16.500 abB	5.2662	14	5.285 aB	5.35549	13	19.461 bA	11.41355	12	17.833 bAB	6.33652	2.960
66 <	15	21.933aD	11.1321	16	0.687 aA	10.6815	19	23.947 bB	12.24506	17	22.294 aC	13.80111	0.360
LSD		7.15			11.174			22.927			1.378		
Total	40	18.15	8.57112	40	10.44	1.65130	40	27.07*	15.06122	40	19.35	9.98088	5.225

E. ALP Level

The results of Table (5) showed significant differences (P ≤0.05) where ALP levels were recorded in patients aged (35-50) were high in both the diabetic nephropathy and nephropathy groups (461.636 ± 164.81and 456.500 ± 241.656, respectively) compared to the diabetic and healthy groups. As for patients aged (51-65), there was a significant increase in diabetic nephropathy patients 503.166 ± 141.621compared to the other groups. In individuals aged (66<), ALP levels of diabetic nephropathy patients were still high (502.88±135.22) compared to the nephropathy and healthy groups. In addition, the overall means for the total sample confirm that ALP levels are markedly higher in diabetic nephropathy compared to the nephropathy, diabetic and healthy groups, reflecting the impact of diabetic nephropathy on kidney function.

Table 5: Serum ALP levels in the control group, diabetes mellitus, nephropathy, and diabetic nephropathy patients across different age categories.

ALP													LSD
Group	Control			DM			N			DN			
	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	
35-50	9	441.888aB	183.492	10	417.400 aC	185.649	8	456.500 aA	241.65678	11	461.636 aA	164.81339	5.137
51- 65	16	323.437 abB	107.711	14	306.000 bB	111.859	13	302.846 bB	80.31069	12	503.166 aA	141.62167	197.729
66 <	15	310.066 bB	172.354	16	371.750 abB	205.581	19	331.210 abB	182.63612	17	502.882aA	135.22152	131.132
LSD		131.822			111.40			153.653			41.245		
Total	40	345.07	157.667	40	360.15	174.149	40	347.05	176.329	40	491.62	143.08518	131.475

F. C Reactive Protein Level

The current study recorded in Table (6) the presence of a significant difference ($P \leqslant 0.05$). Note that there is an increase in CRP levels for diabetic nephropathy patients (60%) who have a positive result compared to the healthy group and other groups, while there are 90% of healthy individuals who have a negative result compared to other groups. In addition, the overall averages of the total sample confirm that CRP levels are significantly higher in diabetic nephropathy patients compared to the healthy group and other groups.

Table 6: Serum C reactive protein Levels in control group, Diabetes Mellitus, Nephropathy, and Diabetic Nephropathy patients Across Different Age Categories

CRP												
Group	Control			DM			N			DN		
	N	Positive	Negative	N	Positive	Negative	N	Positive	Negative	N	Positive	Negative
35-50	9	2 (10.0%)	18 (90.0%)	10	3 (15.0%)	17 (85.0%)	8	3 (15.0%)	17 (85.0%)	11	3 (15.0%)	17 (85.0%)
51- 65	16	2 (10.0%)	18 (90.0%))	14	4 (20.0%)	16 (80.0%)	13	4 (20.0%)	16 (80.0%)	12	3 (15.0%)	17 (85.0%)
66 <	15	3 (15.0%)	17 (85.0%)	16	6 (30.0%)	14 (70.0%)	19	5 (25.0%)	15 (75.0%)	17	12(60.0%)	8 (40.0%)
Total	40	7(35.0%)	53(88.33%)	40	13(65.0%)	47(78.33%)	40	12(60.0%)	48(80.0%)	40	18(90.0%)	42(70.0%)

G. Iron Level

The current study recorded in Table (6) the presence of a significant difference ($P \leqslant 0.05$). Note that there is an increase in CRP levels for diabetic nephropathy patients (60%) who have a positive result compared to the healthy group and other groups, while there are 90% of healthy individuals have a negative result compared to other groups. In addition, the overall averages of the total sample confirm that CRP levels are significantly higher in diabetic nephropathy patients compared to the healthy group and other groups.

Table 7: Serum iron levels in the control group, diabetes mellitus, nephropathy, and diabetic nephropathy patients across different age categories.

Iron													LSD
Group	Control			DM			N			DN			
	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	
35-50	9	107.777 aA	28.239	10	101.200 aA	31.73	8	99.750 aA	35.02	11	97.181 aA	30.39	10.59596
51- 65	16	93.312 bA	14.759	14	88.071 bA	19.08	13	90.692 aAB	21.81	12	82.250 aB	20.53	11.06250
66 <	15	97.133 abA	17.943	16	93.7500 abAB	18.66	19	91.894 aAB	18.59	17	81.647 aB	28.29	15.48627
LSD		14.466			13.1285			9.0576			15.53476		
Total	40	98.000	19.857	40	93.625	22.65	40	93.075	23.194	40	86.100	27.055	11.90

H. Calcium Level

The results of Table 8 showed a slight change in calcium levels among diabetic nephropathy patients in the age groups 35-50, 51-65, and <66 compared with the other groups. In addition, the overall averages of the entire sample confirm a slight change in calcium levels among diabetic nephropathy patients compared with the other groups.

Table (8): Serum calcium levels in the control group, diabetes mellitus, nephropathy, and diabetic nephropathy patients across different age categories.

Ca													LSD
Group	Control			DM			N			DN			
	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	
35-50	9	8.624 aA	0.75125	10	8.323 bA	0.89201	8	8.475 aA	0.792	11	7.612 aA	1.320	1.01172
51- 65	16	8.472 aA	0.80852	14	8.357 bA	0.80834	13	8.392 aA	0.911	12	7.866 aB	1.167	0.60583
66 <	15	8.633 aA	0.90921	16	8.568 aAB	0.75164	19	8.552 aAB	0.692	17	7.761 aB	1.270	0.79146
LSD		0.16083			0.0341			0.16032			0.25394		
Total	40	8.56	0.81857	40	8.43	0.79463	40	8.48	0.771	40	7.75	1.226	0.68125

IV. Discussion

A. Serum Proteins(Total Protein and Albumin)

In our study of patients diagnosed with diabetes, total protein levels were significantly increased in diabetic patients compared to healthy controls (Table 1). Albumin levels were also lower in diabetic patients compared to healthy controls (Table 2) , and these results are consistent with those of other researchers [16-17]. Nazki et al. (2017) showed that total protein concentrations were significantly increased in diabetic patients compared with controls. Competition between serum albumin and hemoglobin may be a factor in the negative correlation between the two and glycation. In addition, it can prevent the glycation of other proteins and modify diabetic complications [17]. As stated by Hardikar et al. (2012), the results suggested that the increase in total protein may be due to an increase in acute phase proteins, globulins, and fibrinogen, as well as a decrease in fractional albumin synthesis rate due to insulin resistance/deficiency. Therefore, low albumin levels in the body due to other causes may lead to an overestimation of HbA1c values in diabetic patients [18].

B. Liver enzymes(ALT, AST,and ALP)

An enzyme called alanine aminotransferase (ALT) catalyzes the conversion of an amino group from alanine to α-ketoglutarate. It is found in various tissues, including skeletal muscle and cardiac tissue, but is primarily concentrated in the cytosol of hepatocytes [19]. Furthermore, hepatocyte lipid buildup, such as that caused by nonalcoholic fatty liver disease (NAFLD), is also reflected by ALT [20].Therefore, it is hypothesized that ALT may raise the risk of microvascular problems associated with diabetes. Its high level in plasma suggests organ failure or tissue injury [21]. Bi et al. (2024) concentrated on ALT's evolving tendency. The average ALT activity in healthy controls was 16.2 IU/L, while patients with diabetic nephropathy had higher ALT levels (21.0 IU/L)[22]. These findings align with the current study's findings (Table 3).

There is an increase in the level of AST in patients with nephropathy and diabetic nephropathy compared to healthy controls (Table 4). These results are consistent with those of the researcher [23-24]. According to Farhan (2014), there was a considerable increase in blood AST levels in patients with diabetic nephropathy, possibly linked to the excessive buildup of amino acids (glutamate) in the serum due to the mobilization of amino acids from protein reserves [23] .Increased enzyme activity results from the conversion of these extra amino acids into ketone bodies for the AST. AST occurs in various organs at lesser activity and is found in high concentrations in the cytoplasm of the liver, kidney, heart, and skeletal muscle. Blood typically has low levels of AST [25].

Serum ALP levels were elevated in both the diabetic and diabetic nephropathy groups compared with healthy controls (Table 5). These results are consistent with [26-27].Zhang et al. (2016) discovered a significant difference between the ALP levels between the blood of healthy groups and the elevated levels of mycotoxin in the blood serum of patients with nephropathy and carriers of toxin. An essential enzyme for bone mineralization in osteoblasts is bone-specific alkaline phosphatase (ALP) [28]. Numerous tissues, including bone, produce ALP. Abnormalities in one of the bone's metabolites, such as chronic renal and diabetic disease, increase estrogenic differentiation, which in turn increases the transit of numerous bound protein compounds, including alkaline phosphatase, and causes a marked rise in blood ALP [29].

C. Inflammatory Markers (C-Reactive Protein - CRP)

By measuring the level of C-reactive protein (CRP) in the group of patients with diabetic nephropathy, it was found that the level of CRP was significantly higher in the patient group compared to the healthy control group, which is consistent with several recent studies that have indicated that CRP levels are higher in patients with diabetic nephropathy compared to healthy individuals [30-31]. According to You et al. (2016), CRP is implicated in the development of numerous diabetic complications, including nephropathy [32]. This finding may be related to the way that CRP causes renal inflammation by inducing a mechanism that is dependent on CD32b and NF-κB. This leads to an increase in T cell and macrophage filtration as well as stimulation of IL-1 beta, TNF-α, and MCP-1 in diabetic renal tubules. Additionally, because CRP promoted TGF-β/Smad signaling, which aggregates extracellular matrix buildup and causes kidney fibrosis in T2DM, and activated the CD32b-Smad3 mTOR signaling pathway [30].

D. Iron and Related Parameters

Table (7) shows lower iron levels in patients with diabetes, nephropathy, and diabetic nephropathy compared to the healthy group. These results are consistent with other researchers [33-35]. Iron plays a crucial role in metabolism, cell growth, proliferation, and the binding and distribution of oxygen. Iron can, however, result in significant oxidative stress and tissue damage due to its capacity to receive and transport electrons [36].The development of functional iron deficiency, a condition in which the body's iron stores are normal or even increased, but the release of iron from them is not rapid enough to ensure normal erythropoiesis, is caused by an increase in inflammatory cytokines, which in turn causes an increase in hepcidin expression. Ferritin levels are either normal or high, and serum saturation is low [37]. By altering the molecular structure of hemoglobin through elevated peroxidation in diabetics, iron shortage speeds up hemoglobin glycation. This could be a result of diabetics' ongoing low-grade inflammation. Additionally, it prolongs red blood cells' lifespan [38].Robles et al., 2018 explained that in cases of diabetic nephropathy, normocytic anemia develops because of insufficient erythropoietin production [34].

E. Calcium Levels

The results of Table (8) showed a slight change in calcium levels in patients with diabetic nephropathy compared to the healthy control group and are consistent with the results of researchers [39- 40]. According to Lim et al. , (2014), in a pooled cohort of patients with CRF stages 3-5, decreased serum calcium was linked to a quicker decline in renal function. The equilibrium of calcium throughout the body is significantly influenced by the kidney. The renal excretion of calcium is changed by abnormalities in the kidneys transport proteins.Reduced intestinal calcium absorption and renal calcium excretion are linked to end-stage renal disease (ESRD), [41-42]. Reduced levels of several calcium- detecting receptors and fat-soluble vitamin D receptors within the parathyroid glands induce hypocalcemia due to hyperphosphatemia linked to CRF. Additionally, a lack of 1, 25-dihydroxycholecalciferol, also known as calcitriol, an active form of vitamin D that facilitates intestinal absorption of dietary calcium [43].Insulin release from pancreatic beta cells requires calcium influx via voltage-gated calcium channels. Serum calcium levels were recognized to be related to the function of pancreatic β cells. Changes in calcium influx can lead to aberrant β-cell function, which in turn can raise the risk of type 2 diabetes [44].

V. CONCLUSIONS

Based on the current study, we conclude that there is an increase in total protein levels and a decrease in albumin levels in diabetic patients. However, depending on the age group, we found an increase in liver enzyme levels, CRP, and calcium in patients with diabetic nephropathy compared to healthy controls. We also conclude that there is a decrease in iron levels in patients with diabetes, nephropathy, and diabetic nephropathy compared to healthy controls. The results showed a slight change in calcium levels in patients with diabetic nephropathy compared to the healthy control group.

CONFLICT OF INTEREST

Authors declare that they have no conflict of interest.

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