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Original Research Article

Evaluation of Myostatin and Some Biochemical Variables in Both Type 2 Diabetic and Diabetic Nephropathy Patients

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Abstract: ***Background:*** The kidneys are essential organs that keep blood pH stable and eliminate excess water, salts, and aid in the body's waste removal. ***Aim of the study:*** The study aims to investigate the importance of myostatin in the development of diabetic nephropathy patients. In addition, the level of urea, creatinine, sodium, potassium, and phosphorus. ***Materials and methods:*** A Case-Control study lasted from- November 2023 to January 2024, at Al-Amal Kidney Dialysis Center and Azadi Teaching Hospital in Kirkuk Governorate. The 135 blood samples, their ages (25-70), were divided into three groups. The first group G1 (n:50) patients with T2DM. The second group G2 (n:55) with diabetic nephropathy. The third group G3 for control. Serum samples were utilized to measure MSTN by ELISA, blood urea, serum creatinine, S.PO_4, S. Na^+, S. K^+, and S. Ca^{++} by spectrophotometer. ***Results:*** Significant variations ($P<0.05$) in MSTN were seen between study groups (8.43±4.69, 10.99±7.05, 5.63±3.38) ng/ml. Additionally, significant differences exist between (G1 vs G2), (G1 vs G3), and (G2 vs G3) with p-value <0.05 Diabetic nephropathy G2 had the highest mean urea and creatinine levels (136.69±38.21, 5.99±1.87), followed by G1 (34.92±9.06, 0.89±0.26) and control group G3 (31.00±4.72), with significant differences ($p \leq 0.001$) among three groups. Additionally, G1 with G2, G2 with G3, and G1 with G3 had significant differences (p-value>0.01). ***Conclusion:*** This study found higher myostatin, urea, and creatinine in diabetic nephropathy and diabetic patients. The data also showed hyponatremia, hypocalcemia, hyperkalemia, and hyperphosphatemia in diabetic nephropathy and diabetic patients.

Keywords: Diabetic nephropathy, Myostatin, Phosphorus, Kidney function test, Electrolyte.

1. INTRODUCTION

Diabetic nephropathy (DN) refers to the existence of a certain glomerular disease pattern. Nephropathy is a medical condition marked by ongoing albuminuria and a gradual decline in kidney function [1]. DN affects a significant proportion, ranging from 20% to 50%, of individuals with diabetes, furthermore, DN is recognized as the primary cause of end-stage kidney disease (ESKD) in numerous communities[2], in the United Kingdom, the proportion of persons beginning renal replacement therapy (RRT) because to DN is 28%, while in the United States it is 44% and in Australia it is 38%, individuals with type 1 (T1DM) or type 2 (T2DM) diabetes who develop diabetic nephropathy (DN) experience significantly poorer outcomes compared to those who do not, DN is frequently linked to high blood pressure and an increased risk of cardiovascular disease and death [3].

In 1997, Colleagues and McPherron discovered a new member of the transforming growth factor (TGF-β) superfamily called growth differentiation factor-8 (GDF-8) in mice [4]. This protein is uniquely detected in both developing and adult skeletal muscle tissue after the GDF-8 gene was disrupted, the researchers saw that the mice exhibited increased muscle mass compared to the mice with the normal GDF-8 gene, the muscles of the mice without GDF-8 weighed 2-3 times more than those of the mice with the normal gene, therefore, it was proposed that GDF-8 primarily acted as a suppressor of skeletal muscle growth and was consequently designated as myostatin (gene name MSTN) [5,6]. Mstn, a

protein, has been linked to various health issues, including age-related sarcopenia, cachexia from chronic renal illness, and heart failure. It also plays a role in energy metabolism, obesity, insulin resistance, diabetic nephropathy, and heart failure, it increases NADPH oxidase in human proximal tubule cells, improving activation and reactive oxygen species (ROS) release, Mstn is also associated with fibrotic regions and tubulointerstitial infiltrates [7].

2. MATERIALS AND METHODS

Study Design

A Case-Control study lasted three months, from- November 2023 to the end of January 2024, at Al-Amal Kidney Dialysis Center and Azadi Teaching Hospital in Kirkuk Governorate, Iraq, it involved collecting 135 blood samples, which were separated into three groups ranging in age from 25 to 75 years. The first group (G1) consisted of 50 patients with T2DM (23 males and 27 females), While the second group (G2) consisted of 55 patients with diabetic nephropathy (27 males and 28 females), They were clinically diagnosed by nephrologists as diabetic nephropathy patients based on their history, clinical examination, renal function tests, and other laboratory tests, the third group (G3) consisted of 30 apparently healthy subjects (16 males and 14 females), All participants in this study provided approved permission with a questionnaire.

Ethical Approval

The research protocol received formal authorization from the Scientific Committee of the Faculty of Medicine at Tikrit University, which had earlier approved the methodology. The Kirkuk Health Department granted clearance for the collection of patient samples.

Exclusion Criteria

1. T1DM
2. Liver failure.
3. Medication.
4. Thyroid disorder (hyper or hypo thyroiditis).
5. Pregnant women.
6. Patients under 18 years old.

Data Collection Form

Upon obtaining informed consent from the participants and gathering their personal data, such as names, ages, weights, heights, BMI, medical history, family history, and duration of illness, each individual was assigned a distinct serial number. Additionally, participants were provided with relevant information about themselves.

Blood Sampling

Five-milliliter blood samples were collected from both the patients and control participants, namely from their forearm veins. Blood sample was transferred into a sterilized plain tube and left to coagulate at room temperature for 20 minutes. The blood was then centrifuged at a speed of 3000 revolutions per minute for 10 minutes to separate the transparent serum. The serum was subsequently transferred to three additional Eppendorf tubes, which were labeled with numbers and stored in a deep freeze at a temperature of -20˚C until they were ready to be analyzed.

The serum sample was analyzed for MSTN using the ELISA method, and for blood urea, serum creatinine, S.PO4, S.Na+, S.K+, and S.Ca++ using a spectrophotometer.

Statistical Analysis

The obtained data were statistically analyzed in the SPSS 26.0 program. Statistical significance was accepted as $P < 0.05$. The data were analyzed statistically according to the Analysis of Variance test(ANOVA)

3. RESULT AND DISCUSSION

The current study showed that there was no significant differences between studied groups according to the Gender. The prevalence of the diseases were high in no smoker patients (76%) for G1 and (78.2%) for G2 compared with smoker ones (24%) for G1 and (21.8%) for G2. Also non-diabetic family history recorded high result (67.3%) for G1 and (84%) for G2 compared with those with diabetic family history. Table (1).

Table 1: The percentage of Gender, smoking and family history in Diabetic Nephropathy patients compared with Diabetic patients

parameters		Diabetes G1 n (50)	Diabetic Nephropathy G2 n (55)	Total n (105)
Gender	Male	23(46%)	27(49%)	50(47.6%)
	Female	27(54%)	28(51%)	55(52.4%)

parameters		Diabetes G1 n (50)	Diabetic Nephropathy G2 n (55)	Total n (105)
Smoking	**Smoker**	12(24%)	12(21.8%)	24(22.9%)
	Non-smoker	38(76%)	43(78.2%)	81(77.1%)
Family history	**Diabetic**	18(32.7%)	8(16%)	26(24.8%)
	Non-diabetic	37(67.3%)	42(84%)	79(75.2%)

The (mean±SD) levels of MSTN in serum of G1, G2, and G3 were in illustrated in table (2). There were significant differences (P<0.05) in MSTN between study groups that were (8.43±4.69, 10.99±7.05, 5.63±3.38) ng/ml respectively. In addition significant difference between (G1 vs G2), (G1 vs G3) and (G2 vs G3) at p-value <0.05.

Table 2: The mean ± SD of MSTN in study groups compared to control group.

Groups		MSTN ng/ml
		Mean± SD
G1 (n:50)		8.43±4.69
G2(n:55)		10.99±7.05
G3(n:30)		5.63±3.38
p-value	G1/G2/G3	<0.001*
	G1/G2	**<0.001***
	G2/G3	<0.001*
	G1/G3	0.005*

* p-value <0.001 highly significant, Mean± SD: Mean standard deviation

Table (3) displays the average standard deviation (SD) of urea and creatinie in the different study groups. The study reveals that the diabetic nephropathy G2 group has the highest average levels of urea (136.69±38.21) and creatinine (5.99±1.87), followed by the diabetic G1 group (34.92±9.06, 0.89±0.26). The control group G3 has the lowest average level of urea and creatinie (31.00±4.72,0.78±0.17), and there is a highly significant difference ($p \leq 0.001$) in urea and creatinie levels among the three groups. Furthermore, there are significant differences (p-value>0.01) in urea and creatinie levels between G1 and G2, G2 and G3, and G1 and G3.

Table 3: The mean ± SD of Urea and creatinine in study groups compared to the control group

Groups		Urea mg/dl	Creatinine mg/dl
		Mean± SD	
G1 (n:50) d		34.92±9.06	0.89±0.26
G2(n:55) n		136.69±38.21	5.99±1.87
G3(n:30) c		31.00±4.72	0.78±0.17
p-value	G1/G2/G3	<0.001*	<0.001*
	G1/G2	**<0.001***	<0.001*
	G2/G3	<0.001*	<0.001*
	G1/G3	0.031	0.042

* p-value <0.001 highly significant, Mean± SD: Mean standard deviation

The present study shows highly decreased Sodium and calcium in diabetic nephropathy patients (135.29±3.22 mEq/dl, 8.38±0.65 mg/dl), followed by diabetic (138.60±3.22 mEq/dl, 9.54±0.43 mg/dl), then control (143.71±3.52, 9.80±0.52) at p-value <0.001. In addition significant increase in Sodium and calcium in diabetic patients as compared with diabetic nephropathy, at p-value <0.001.

The current investigation demonstrates a significant elevation in K+ and PO_4 levels in patients with diabetic nephropathy (4.75±0.64, 5.54±1.01) mg/dl, followed by diabetic patients (4.10±0.36, 3.24±0.59) mg/dl, and then control subjects (3.88±0.54, 3.01±0.30) mg/dl, with a p-value of <0.001. Furthermore, there was a substantial elevation in the levels of potassium (K+) and phosphate (PO4) in diabetic patients compared to those with diabetic nephropathy, with a p-value of less than 0.001. Diabetic individuals exhibit higher levels of K+ and PO4 compared to the control group, with a statistically significant difference at a p-value of less than 0.05. as shown in Table (4).

Table 4: The mean ± SD of (Sodium) in study groups compared to the control group

Groups	Sodium mEq/dl	Ca^{+2} mg/dl	K^{+} mg/dl	PO4 mg/dl
	Mean± SD			
G1 (n:50)	138.60±3.22	9.54±0.43	4.10±0.36	3.24±0.59
G2(n:55)	135.29±3.22	8.38±0.65	4.75±0.64	5.54±1.01

Groups		Sodium mEq/dl	Ca^{+2} mg/dl	K^{+} mg/dl	PO4 mg/dl
		Mean± SD			
G3(n:30)		143.71±3.52	9.80±0.52	3.88±0.54	3.01±0.30
p-value	G1/G2/G3	<0.001*	<0.001*	<0.001*	<0.001*
	G1/G2	**<0.001***	<0.001*	<0.001*	**<0.001***
	G2/G3	<0.001*	<0.001*	<0.001*	<0.001*
	G1/G3	<0.001*	0.0179	0.0318	0.0508

* p-value <0.001 highly significant, Mean± SD: Mean standard deviation

4. DISCUSSION

Diabetes totally reverses the sex-gender difference, resulting in women bearing a larger burden of cardiovascular problems. Moreover, all risk factors linked to diabetes seem to have a greater impact on females with diabetes compared to males. Sex-gender disparities in diabetes can be attributed to both genetic and hormonal variables. Women may tend to be diagnosed with diabetes later and in more severe situations. The study's authors disclosed that diabetic nephropathy is a highly significant consequence of both type 1 (T1DM) and type 2 diabetes mellitus (T2DM). Studies have shown that there are variations between genders in the occurrence and frequency of DKD, its distinct forms and symptoms, and various risk factors, which affect each gender differently. The discrepancies can be mostly attributed to hormonal variables, particularly the decline in estrogen levels. Furthermore, researchers have examined the effects of sex chromosomes and the role of gene-sex interactions on numerous susceptibility genes for DN [9]. Prior research has demonstrated that smoking is regarded as a distinct risk factor in the onset and advancement of diabetic nephropathy. Smoking contributes to the development of diabetic nephropathy by various factors, such as oxidative stress, hyperlipidemia, deposition of advanced end glycation products, and glomerulosclerosis [10]. Zhang *et al.,* [11] found no notable variations in age, gender, duration of diabetes, smoking habits, hypertension, or coronary heart disease among patients with diabetic nephropathy. The study conducted by Wang *et al.,* [12] found that there is a strong correlation between a family history of diabetes in first-degree relatives and a quick reduction in estimated glomerular filtration rate (eGFR) in relatively young patients.

Myocytes produce and secrete the myokine called myostatin, which hinders the growth of muscle cells by limiting their cellular proliferation. A study conducted in Baghdad city [13] found that the group with metabolic syndrome showed a considerable rise in myostatin levels compared to the control group. The research conducted by Rasheed *et al.,* [14] revealed that the levels of myostatin in patients were higher compared to the levels in the control group (6.303 vs 0.313). These findings align with the current investigation, which demonstrated that the myostatin levels in diabetes patients were measured at 8.43. Myostatin (MSTN) is a member of the transforming growth factor (TGF)-β superfamily. The study conducted by Verzola *et al.,* [15] demonstrated that MSTN is present in the human kidney and is particularly abundant in DN, primarily in the tubule-interstitial region. The study also revealed that MSTN plays a significant role in activating the proximal tubules and proposes that the overexpression of MSTN contributes to the development of kidney interstitial fibrosis in DN.

The study found that the group with diabetic nephropathy had the highest levels of urea and creatinine, followed by the group with diabetes, which also showed elevated concentrations of urea and creatinine compared to the control group. This is due to impaired renal clearance, resulting in the accumulation of urea in the blood. This investigation corroborates the findings of a study conducted in Kirkuk by researchers [16,17]. Urea is a metabolic byproduct generated by the body during the breakdown of dietary protein. If you experience a decline in renal function, your kidneys may become unable of fully eliminating urea from your bloodstream [18]. Urea is commonly used to assess renal function, but its levels only rise dramatically when a serious kidney injury has occurred, and there is a delay in its growth. Creatinine is a metabolic byproduct produced by skeletal muscles. Typically, the kidneys eliminate it from the blood and excrete it in urine. Impaired kidney function results in the accumulation of creatinine in the bloodstream. Serum creatinine is excreted through glomerular filtration, and its levels rise in the presence of kidney disease. Currently, it serves as the main diagnostic test for renal disease [20]. Creatinine is the primary indicator used to evaluate glomerular filtration rate (GFR). However, its ability to detect early stages of kidney damage is limited. By the time an increase in creatinine levels is noticeable, a major decrease in GFR has already occurred [21]. Mistry *et al.,* [22] demonstrated that patients with diabetic nephropathy exhibit elevated levels of serum creatinine .

This results showed that there was hyponatremia, hypocalcemia, hyperkalemia, and hyperphosphatemia in both groups diabetic nephropathy, and diabetic patients as compared with control, but this condition highly appearance in diabetic nephropathy than diabetics may be due to correlated electrolyte by kidney function test which is produced by a wide range of factors that lead to the reduction of the kidney's effective functional unit, resulting in chronic kidney disease (CKD). Al-obaidi *et al.,* [23] Showed hyperkalemia, hypocalcemia, and hyponatremia in diabetes nephropathy and non-diabetic nephropathy as compared with the control group, while non-significant differences in the serum levels of k+, Na+ and Ca+2 in G1 compared to G2 group. The occurrence of dyselectrolytemia, which encompasses hyponatremia, hyperkalemia, and hypocalcemia, is a frequent characteristic of chronic kidney disease (CKD) in both diabetic and non-

diabetic CKD patients [24]. The electrolyte imbalances are a result of the decline in kidney function, which is produced by a wide range of factors that lead to the reduction of the kidney's effective functional unit, resulting in chronic kidney disease (CKD) [25]. The calcium imbalance and hypocalcemia in both diabetic and non-diabetic chronic kidney disease (CKD) are primarily caused by uraemic bone disease. This condition generally manifests early in CKD patients due to hypophosphatemia, which is associated with CKD. Additionally, there is a decrease in the number of calcium-detecting receptors and vitamin D receptors in the parathyroid glands. Additionally, there is a lack of 1, 25 dihydroxycholecalciferol, which is the biologically active form of vitamin D that facilitates the absorption of dietary calcium from the gastrointestinal tract [24]. The study done by [26] shows hyperphosphatemia in diabetic nephropathy. Recently, reported that an elevation of extracellular phosphate increased the production of reactive oxygen species in bovine aortic endothelial cells, suggesting that hyperphosphatemia may be involved in endothelial dysfunction and insulin resistance resulting from oxidative stress [27].

5. CONCLUSION

Based on the findings of this study, it may be inferred that individuals with diabetic nephropathy and type 2 diabetes mellitus (DM2) exhibit elevated levels of myostatin. This study concluded an increase, in urea, and creatinine in both groups diabetic nephropathy, and diabetic patients. As well these results showed that there was hyponatremia, hypocalcemia, hyperkalemia, and hyperphosphatemia in both groups of diabetic nephropathy, and diabetic patients

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