

FULL PAPER

The role of metalloendopeptidase (MEP) as a vital predictor of early diabetic nephropathy and its relationship to some other biochemical variables

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Metalloendo peptidase is a neutral endopeptidase that cleaves peptides at the amino side of hydrophobic residues and inactivates several peptide hormones, including atrial natriuretic factor, giucagon, enkephalin, substance p, neurotensin, oxytocin, and bradykinin. It is also a major enzyme for the degradation of beta-amyloid. This study aimed to measure enzyme activity and compare it with other biochemical changes in sera patients with diabetic nephropathy. The study included 35 pathological samples of people with diabetic nephropathy, 24 samples from males and 11 samples from females, as well as the same number of healthy people as a comparison group of 15 males, 20 females, with the ages of both groups of patients with diabetic nephropathy and the healthy ranging between (60-35) years. When compared with healthy controls, a significant change (P 0.001) was seen in each of Age, SBP, DBP, FBS, HbA1c, HOMA, LDL, VLDL, TAG, TC, urea, creatinine, and uric acid over the period of September 2020 - December 2020. While there was a substantial drop (p 0.001) in both insulin and HDL when compared with the control group, there was no significant change (P 0.331) in BMI. As for the enzyme and cystatin-C, a significant change (P <0.001) was observed in the serum of patients with diabetic nephropathy compared with healthy subjects. Also, there was a positive correlation relationships between the enzyme and each of cystatin - C, Age, SBP, insulin, HOMA, HbA1c, FBS, DBP, creatinine, urea, and uric acid while a negative correlation between the enzyme and BMI, DBP, TC, TAG, VLDL, HDL, and LDL was observed. We can conclude from the results that the enzyme activity increases significantly in the serum of patients with diabetic nephropathy compared with healthy controls. This is due to damage to the kidney's tissues and cells, which results in the enzyme's release into the bloodstream and increased activity there. Because the enzyme is prevalent in renal tissues. It should be used as a clinical variable in patient diagnosis as an early predictor of disease.

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Introduction

Diabetic nephropathy (DN) or diabetic kidney disease (DKD) is a common complication of diabetes mellitus type 2. The mechanism

involved includes changes in blood vessels that supply peripheral nerves and metabolic disorders. Diabetic nephropathy is characterized by increased protein excretion, mainly albumin in urine, a decline of the

glomerular filtration rate (GFR), and elevated blood pressure leading to end-stage renal failure. Therefore, early diagnosis of diabetic nephropathy is essential, and early therapy decreases the progression of renal disease [1]. It is essential to note that only 30% to 40% of patients with diabetes develop diabetic nephropathy [2]. Specific treatment of patients with diabetic nephropathy can be divided into 4 significant arenas: Cardiovascular risk reduction, glycemic control, blood pressure control, and inhibition of the renin-angiotensin system (RAS) [3].

The severity of glomerular damage is proportional to GFR value, diabetes Mellitus DM duration, and blood glucose regulation. The main pathophysiological changes in diabetic nephropathy include the thickening of the glomerular basement membrane (GBM), mesangial expansion, nodular sclerosis – Kimmelstiel-Wilson change, diffuse glomerular sclerosis, tubular interstitial fibrosis, and arteriosclerosis and hyalinosis of kidney blood vessels [4,5].

Nephrilysin (NEP) is a ubiquitous zinc-dependent membrane metalloendopeptidase (EC 3.4.24.11) that is expressed in multiple organs such as the kidneys, lungs, endothelial cells, vascular smooth muscle cells, cardiac cells, fibroblasts, neutrophils, adipocytes, testes, and brain [7,6].

Cystatin C is a 13 kDa protein with low molecular weight produced by all nucleated cells and freely filtered by the renal glomeruli and reabsorbed in the proximal tubule. Cystatin C is not affected by age or muscle mass in a healthy person. Increased urinary cystatin C is a marker of renal tubular dysfunction. Cystatin C regulates the immune system and extracellular proteolysis [8,9].

This study aimed to measure enzyme activity and compare it with other biochemical changes in sera patients with diabetic nephropathy.

Materials and methods

In this study, 35 blood samples were collected from patients with diabetic nephropathy and healthy individuals from Yarmouk Teaching Hospital in Baghdad - Iraq. The ages of the two groups ranged between (35-60) years from (September 2020 - December 2020).

Biochemical variables such as age, height, weight, family history, and BMI of all participants were noted. Blood samples were taken for laboratory investigation, including cystatin C Kit. FBS, TC, TG, HDL, LDL, and VLDL were measured using an automated analyzer (PT TURKEY). Glycated Hemoglobin (HbA1c) was estimated using HPLC. The concentration of creatinine (Biolabo-France), urea (Camthch Medical), and uric acid (Biolabo-France) were also determined. Because the enzyme is prevalent in renal tissues, it should be used as a clinical variable in patient diagnosis as an early predictor of disease.

Results

Table 1 shows the physical measures and clinical features of diabetic nephropathy patients compared with healthy controls. A significant change was observed in both age and hypertension in patients with DN compared with healthy subjects. At the same time, there was a significant change in BMI of patients compared to healthy controls. The results showed that DN patients had higher mean of TC, TG, LDL-C, VLDL-C, glucose, and HbA1c levels than healthy people. At the same time, the results showed that the average level of HDL-C and insulin level decreased significantly in the serum of patients with DN compared with healthy subjects. The average amount of urea, creatinine and uric acid in the blood serum of DN patients increased significantly (P 0.001) compared with the healthy control group.

TABLE 1 Anthropometric and clinical features of the studied group

Parameters	Mean \pm SD		P-value
	DN N (35)	Control N (35)	
(Male/Female) Number	(24/11)	(15/20)	-
Age (year)	48.11 \pm 4.10	37.49 \pm 4.348	0.001
BMI (Kg/m ²)	27.26 \pm 3.381	28.12 \pm 3.91	0.331
SBP (mmHg)	14.26 \pm 1.121	12.37 \pm 0.731	0.001
DBP (mmHg)	8.8 \pm 0.72	8.26 \pm 0.657	0.002
Family history (Positive/negative)	(29/6)	(6/29)	-
FBS (mg/dl)	181.97 \pm 43.897	86.77 \pm 6.117	0.001
HbA1c (%)	8.1 \pm 1.433	4.94 \pm 0.377	0.001
Insulin (μ u/mL)	12.12 \pm 2.622	15.78 \pm 2.004	0.001
HOMA ()	5.31 \pm 1.107	3.39 \pm 0.480	0.001
Tc (mg/dl)	199.74 \pm 18.405	160.71 \pm 18.175	0.001
TAG (mg/dl)	139.69 \pm 25.52	116.57 \pm 22.039	0.001
VLDL (mg/dl)	27.94 \pm 5.104	23.314 \pm 4.408	0.001
HDL-C (mg/dl)	47.83 \pm 4.253	50.43 \pm 5.559	0.031
LDL-C (mg/dl)	124.21 \pm 17.923	85.43 \pm 12.31	0.001
Urea (mg/dl)	55.43 \pm 7.35	27.06 \pm 2.543	0.001
Creatinin (mg/dl)	1.15 \pm 0.25	0.7 \pm 0.08	0.001
Uric acid (mg/dl)	6.98 \pm 1.411	4.47 \pm 0.559	0.001

Table 2 displays the rate of activity of metalloendopeptidase in diabetic nephropathy patients and healthy controls. When performing a comparison statistically, it was found that there were significant

differences between the activity of the enzyme (MEP) in patients compared with the control group with a probability level ($P < 0.001$). Thus, in general, the activity of the enzyme increases in patients with diabetic nephropathy.

TABLE 2 Serum Metallo-endopeptidase activity in DN and control groups

Parameters	Mean \pm SD		P-value
	DN N (35)	Control N (35)	
Metallo-endopeptidase (I.U/L)	21.57 \pm 6.301	9.67 \pm 3.622	0.001

Table 3 shows that the mean level of cystatin C significantly increased ($P < 0.001$) in

the blood serum of patients with DN compared with the healthy as a control group.

TABLE 3 Serum cystatin C levels in DN and control groups

Parameters	Mean \pm SD		P-value
	DN N (35)	Control N (35)	
Cystatin C (ng/mL)	1.72 \pm 0.116	1.49 \pm 0.054	0.001

We investigated the association between MEP activity and biochemical variables. The correlation coefficient was discovered (cystatin C, age, body mass index, hypertension, hypotension, fasting blood glucose, HbA1c, insulin, HOMA, TC, TAG, VLDL, HDL-C, LDL-C, urea, creatinine, and urea acid, etc. As displayed in Table 4, the linear relationship of MEP enzyme with cystatin C, age SBP, FBS, HbA1c, insulin, HOMA-IR, urea, creatinine, and uric acid for serum of diabetic

nephropathy patients is positive as indicated by coefficient values linear correlation (r). The results also showed that the linear relationship between the enzyme and both (BMI, DBP, TC, TAG, VLDL, HDL-C, LDL-C) in the serum of diabetic nephropathy patients was negative.

Table 4 shows linear correlation coefficient between MEP and some biochemical variables in blood serum of patients with DN.

TABLE 4 The correlations between Metallo endopeptidase and some biochemical parameters

Parameters	R	P	Parameters	R	P
Cystatin C (ng/mL)	0.710	0.001	TAG (mg/dL)	- 0.354	0.037
Age (years)	0.020	0.908	VLDL (mg/dL)	- 0.354	0.037
BMI (kg/m ²)	- 0.378	0.025	HDL-C(mg/dL)	- 0.233	0.178
SBP (mmHg)	0.034	0.845	LDL-C (mg/dL)	- 0.031	0.859
DBP(mmgH)	- 0.056	0.748	Urea (mg/dL)	0.085	0.626
FBS (mg/dL)	0.055	0.754	Creatinine (mg/dL)	0.401	0.017
HbA1c (%)	0.046	0.793	Uric acid (mg/dL)	0.231	0.181
Insulin (μu/mL)	0.152	0.384			
HOMA	0.261	0.129			
Tc (mg/dL)	- 0.164	0.345			

Discussion

DN is one of the common complications of diabetic patients. It is also one of the important reasons for reaching the end stage of kidney disease (ESRD) globally and is widespread in our society with the rise in diabetes and the occurrence of complications, which is one of the most critical risk factors for accelerating the incidence of diabetic nephropathy. Moreover, among essential factors known as risk factors that cause diabetes complications are gender, age, blood pressure, and body mass index (BMI) [10,11].

Decades ago, it was found that the prevalence of type 2 diabetes was increasing rapidly in the world, especially in low and middle-income countries. The complications of type 2 diabetes and the pathological conditions are affected by the quality of life in people with type 2 diabetes. The nature of poor life leads to a high mortality rate [12].

Ages over 40 are a risk factor for type 2 diabetes, consistent with previous studies [13,14]. Furthermore, Masanori *et al.* (2001) reported that there was no significant change in sex or BMI in diabetic nephropathy patients, similar to the current study's findings [15]. High blood pressure (SBP) is an essential factor that plays a significant role in predicting DN incidence, as previous have studies found a strong relationship between high blood pressure and diabetic nephropathy in patients with type 2 diabetes. It was found that SBP was associated with an increased risk of albuminuria [16]. It is evident from Table 2

that the activity of the enzyme metalloandopeptidase increases in the serum of patients with DN, as the mean \pm standard deviation of the enzyme was (21.57 \pm 6.301) (I.U/L), while the mean \pm standard deviation of the enzyme was (9.67 \pm 3.622) (I.U/L). In healthy blood serum. Previous studies have indicated an increase in the activity of the enzyme metalloendopeptidase in cases of heart disease and obesity. Insulin resistance and high blood pressure are affected by these diseases [17]. The explanation is the rise in oxidative stress processes that contribute to protein degradation as the disease duration grows. The high activity of the enzyme is consistent with the results of both Vendemial (1999) and Zitta *et al.* (2003) [18,19]. The increased activity of the enzyme in diabetic nephropathy patients' serum may be related to the enzyme's abundance in kidney tissues and damage to the kidney tissues causing enzyme release into the circulation, according to Sansoe *et al.* (2006). Finally, Alloush *et al.* (2011) indicated a high activity of the enzyme in patients with renal impairment, and the literature did not indicate the existence of a previous study explaining the relationship of the enzyme with DN. The results showed that the enzyme is clinically crucial in diagnosing diabetic nephropathy, so it is recommended to adopt it as one of the clinical variables used in diagnosing the disease. In addition, the proportion of kidney damage grows as the efficacy of the enzyme increases with the percentage of kidney damage. This is the release of the enzyme through the kidney

membrane due to its injury. The enzyme has clinical importance in other diseases such as heart diseases, blood pressure, and diabetes.

The results show that glucose levels increased significantly, consistent with previous results [15]. The cause of the increase in glucose concentration is due to hypertension resulting from enlargement of the left side of the heart [20]. The cause of the high glucose level is also owing to an imbalance in blood sugar balance. This is attributable to insulin production and secretion [21] or a lack of glucose intake receptors in cells. It is also believed that this imbalance is because genetic factors or acquired factors result from the decrease or lack of insulin level, leading to high blood sugar [22,23].

This gland, however, will eventually be unable to meet the increased demand for this hormone, resulting in an increase in blood glucose levels and clinical signs such as a weak cell response to the hormone insulin or a defect in insulin function. The pancreas gland in the initial stages of injury compensate resistance to the insulin hormone in the tissues by increasing the amount of insulin produced. The increased demand for this hormone causes an increase in blood glucose levels and clinical signs such as a weak cell response to insulin or a defect in insulin action [25].

Glucose hemoglobin is one of the primary routine tests used as a vital marker for long-term blood glucose control. According to this property that this test possesses, HbA1C can predict the risk to diabetic patients through complications. For example, the increase in HbA1c levels in patients with DN indicates poor blood sugar control, leading to an increase in blood viscosity [26].

In addition to an increase in blood glucose, increased glucose hemoglobin levels tend to influence red blood cells (RBC) characteristics by decreasing RBC flexibility and increasing RBC breakdown, increasing blood viscosity. Also, an increase in HbA1C levels can lead to a

change in permeability. This is because the lipoprotein membrane interferes with RBC and thus leads to a change in viscosity. According to the findings of Lin *et al.* (2014), there is a direct link between HbA1C and insulin resistance IR using a traditional glucose tolerance test. Furthermore, HbA1c has a strong connection with insulin sensitivity in healthy persons. As a result, HbA1C is one of the excellent biomarkers for insulin resistance to test diabetic patients or the onset of disease in obese people [27]. Also, patients with high lipids have an elevated glucose hemoglobin level [28].

The results of the current study are in line with those of Alicia *et al.* (2003). Through biological tests, the current study results showed that the fasting blood glucose levels and the homeostasis model assessment (HOMA-IR) were significantly higher in patients with DN which may be due to an increase in the level of both glucose. The fat and stress caused by the endoplasmic reticulum and the apoptosis of the cells all lead to the gradual loss of B-Cells 64P [29]. The occurrence of insulin resistance and the defect in insulin secretion by the pancreatic B-cells are associated with the emergence of type II diabetes [30] Cardiomyopathy, neuropathy, and nephropathy result. Inventiveness may explain insulin resistance. Insulin resistance has various pathophysiological factors (31). The occurrence of insulin resistance is one of the distinguishing features of type 2 diabetics as it affects fat and muscle cells. The increase in kidney disease and the appearance of its signs is also linked to insulin resistance. Insulin resistance also increases in diabetic nephropathy patients, which is called renal insulin resistance. This resistance is due to insulin being affected by high levels of free fatty acids (FFA), having been proven by previous studies similar to the results of the current study [32]. Numerous studies have also demonstrated that high dosages of insulin generate a significant drop in blood sugar. Reducing renal filtration rate and increasing

albumin release in urine, causing several kidney issues and eventually leading to kidney failure [33].

The high level of lipids in the blood is accompanied by a gradual development of diabetic nephropathy and complications in other blood vessels. In addition, the reduction of fats may delay kidney damage [34]. However, earlier studies have shown that high lipid levels in diabetic patients are the most common. Especially when patients developed DN, the high level of TC with TAG caused a substantial shift in patients compared to healthy, consistent with past findings [10].

According to Abdul-Rammam (1995) and Akbar (2001), the incidence of high blood lipid levels is between 25-60 % of diabetic patients. This is in addition to the presence of similar results. Studies have confirmed that the rates of lipids and lipoproteins range between 25% and 50% for TAG and total cholesterol, respectively [10].

Increased triglycerides (TG) are associated with an increased risk of type 2 diabetes, as high blood sugar results in oxidative stress, causing active oxygen species (ROS) production, damages cell membranes, and inactivates antioxidant enzymes. Also, high levels of total cholesterol and triglycerides can increase the chances of developing diabetic nephropathy, mainly when fat accumulates in the kidney tissues of Type II patients. In addition, previous studies consistent with the current study results indicated an increase in TC, TAG, and FFA, leading to their storage in the kidney tissues, which increases oxidative stress and, consequently, impaired renal function [36].

In addition to high triglycerides with high blood pressure, glomeruli lead to fatty deposits in the glomerular vascular tube [37]. It causes the eventual development of DN and the significant expansion of the kidney pathways. Previous research has established elevated lipid levels in the blood of diabetic patients with DN. Diabetes Mellites are a significant risk factor for heart disease [38].

The most common examples of this are patients who have elevated TG levels and low HDL-C levels. Our findings align with prior research showing a higher prevalence of elevated lipids in diabetic patients, especially those with DN, than in people with diabetes only [39].

Several studies have confirmed that a rapid decrease in the GFR rate in patients with DN is due to high cholesterol in the blood. Furthermore, individuals with DN frequently have metabolic problems that result in high VLDL-C, LDL-C, and low HDL-C, which is consistent with past findings [36].

This decrease in HDL-C level may be attributed to an increase in the generation of active oxygen varieties that leads to an increase in total cholesterol or a decrease in the level of HDL-cholesterol in the blood [41,40]. The increase in sugar and insulin resistance leads to a decrease in HDL, which stimulates the activation of the hormone lipase. This causes an increase in the synthesis of triglycerides in the liver and their excretion in the fatty tissues and increases the flow of free fatty acids into the body tissues [42]. Moreover, oxidation of LDL-C is the destruction of cholesterol. In the body, due to the active oxygen varieties, it reduces the level of HDL-C. HDL-C is the basis for the process of transporting cholesterol from the body cells to the liver [43]. As a result, it reduces the cholesterol present in the blood vessels. The low level of HDL-C is also due to several factors, the most important of which is the high level of oxidation and the effectiveness of the enzyme, cholesterol ester transferase, which transfers the cholesterol ester from the HDL to VLDL-C, leaving HDL-C rich in triglycerides or less to Apo-A so that it remains free, facilitating its filtering from the kidneys [45,44]. Or, the reason may be due to the increase in LDL-C concentration and the speed of its oxidation due to the increase in the number of free radicals produced, leading to a decrease in the concentration of HDL-C in the blood serum [47,46]. The results showed that

the mean level of LDL-C was significantly higher ($P < 0.001$) in the serum of patients with DN compared with healthy subjects. This is in agreement with the results of Jisieike *et al.* (2011). They indicated the role of fats in causing kidney damage through different physiological mechanisms. However, the high level of lipids in the blood increases the susceptibility to developing diabetic kidneys or reaching End-stage Renal disease (ESRD). According to [48] Moorhead *et al.* (1982), high blood lipid levels accelerate gradual renal function decline and, eventually, chronic kidney disease by allowing for more efficient transport of lipids and lipoproteins to the renal mesangium. Mechanical details that cause lipid damage to the renal glomerulus are not fully understood [48]. Lipoproteins are absorbed into the glomerulus via specific receptors in the mesangium cells (the glomerulus' structural unit) and the glomerular epithelial cells [49].

Moreover, its increased concentration causes oxidative stress, which will lead to the transformation of LDL-C To the LDL-modified. This, in turn, means that the LDL modified has two types: Glycosylated and oxidized LDL. When modified LDL is accumulated in the mesangium, it will convert monocytes into macrophages [50]. Within Mesangium cells, the concentration of modified lipoproteins stimulates the release of various molecules referred to as chemotactic factors. These factors have led to the infiltration of monocyte cells [51], which play an essential and significant role in causing the emergence of glomerulosclerosis and tubular fibrosis, especially in patients with nephropathy diabetes. When the macrophage increases, this will stimulate the release of free radicals or active oxygen species and the release of cytokines, which will produce extracellular proteins, thus promoting mesangial expansion. This expansion causes renal damage as it is the case in patients with diabetic nephropathy.

As it is known, LDL-C is a major transporter of cholesterol from the liver to peripheral tissues. It contains a high percentage of cholesterol, as it is noted that its high level may lead to atherosclerosis [52]. This leads to LDL oxidation by ROS into OX-LDL, which disrupts the endothelial cell function and causes impaired metabolism. The progression of DN impairs lipoprotein lipase LPL [53] activity in endothelial cells, increasing LDL levels, or it may be due to the increase in the level of malondialdehyde resulting from oxidative stress [54] or the oxidative stress resulting from the free oxygen radical as these two hormones activate the enzyme lipase, which is sensitive to the two hormones. They are sensitive to triglycerides. In the fat cells, causing rapid degradation of TAG and liberation of fatty acids, and ultimately increasing LDL-C with the progression of DN[55], patients are exposed to double oxidative stress due to the weakness of antioxidants (vitamin C, vitamin E, etc.) and the increase in free radicals formed in the endothelial cells in response to inflammation formed by lipid peroxides and the increased activity of oxidative factors, which increases tissue kidney damage and speeds up the progression to the stage of Renal Failure. The results showed that the mean level of VLDL-C increased significantly ($P < 0.001$) in the serum of the group of patients with DN compared with healthy subjects. This is consistent with the results of previous studies that have confirmed that an elevated level of VLDL-C is associated with diabetes. These studies also suggest that there may be a reduction in metabolic processes and impaired excretion of metabolic wastes, which is exacerbated by poor control of sugar, insulin resistance, and high blood pressure. The apparent development of DN has a close relationship with the elevation of lipid levels in the blood. Suppose it is found that the percentage of triglycerides in VLDL is significant, and for this reason, the increase in its concentration is due to the increase in oxidation in the body.

Furthermore, this reduces the activity of the Lipoprotein lipase present (LPL) in various tissues of the body, which causes an imbalance in lipid levels and an increase in the level of TAG and thus a High level of VLDL in the blood [57].

The study results are consistent with those of results of katzung [58] in the blood serum of people with type 2 diabetes, as the high level of urea is due to the loss of glucose. As a result of the lack of insulin and the reliance on protein as an alternative source of energy results in the development of urea, it is a source of direct energy in the body [59]. The increase in urea is also due to the existence of a relationship between diabetes mellitus and nephropathy. Urea is considered an indicator of the renal filtering function as it predicts diabetic nephropathy in patients with diabetes due to high blood sugar concentration [60]. The high level of urea in the blood serum of diabetics is affected by the length of the disease. Lack of control over it leads to severe complications such as DN. In addition, the decreased efficiency of the kidneys results from an imbalance in the effectiveness of some hormones such as angiotensin or imbalance of the number of nephrons or imbalance of the filtration area, which has a lot to do with the effectiveness of glomeruli [61].

The kidneys purify the blood from creatinine and excrete it with the urine in a healthy person. However, in a pathological situation, one of the most important causes of elevated creatinine levels is the damage to the kidney tissues and their incapacity to perform their tasks adequately. This is consistent with the results of the current study [62]. High blood pressure increases pressure on the walls of blood vessels around the kidneys, causing damage and thus impairing their functions [66]. Furthermore, oxidative agents and free radical damage that produce functional disturbance of the cells of the inner layer of the glomerular capillaries may be the reason for the rise in creatinine levels. It leads

to an increase in the concentration of creatinine in the blood and a decrease in its excretion in the urine [63].

This is in addition to other factors that affect the level of creatinine in the blood serum, such as age, gender, and a person's physical condition [64]. A previous study has found that uric acid affected diabetic nephropathy patients. In type 2 diabetes individuals, uric acid has been a separate risk factor for renal impairment. The action of uric acid on renal blood vessels in diabetic patients causes kidney damage and function decrease in addition to high uric acid and its influence on the glomeruli that may cause it to grow and harden [65].

Cystatin C is one of the essential biomarkers of early prediction. DN in Type 2 Diabetes Mellitus Cystatin C is a small essential protein with a molecular weight of 13 KD. It belongs to the family of statins produced by eukaryotic cells and is filtered freely through the glomeruli-reabsorption catabolism through the tubules.

Table 3 shows that the stable production of cystatin C strongly indicates that GFR is the primary determinant of levels of cystatin C in the blood serum. Previous studies that corroborate the current study's findings have indicated that statin C is a more sensitive biomarker of early degradation of the glomerular filtration rate of creatinine because it provides an early indication for individuals with DN and patients with chronic glomeruli [63]. Another study indicated that the average levels of statin C in the serum of individuals with DN in the third stage of the disease were significantly higher than those detected in the first and second stages. It was found that there is a relationship between levels of cystatin C and glomerulitis. Chronic kidney disease is more common in DN [65,66]. In comparison to serum creatinine levels, cystatin C was statistically more linked with the predictive phase of DN with immunoglobulin (IgA), as cystatin C levels increased in individuals with chronic

glomeruli; the initial stage of diabetic nephropathy. The disease consists of five stages. In patients with a variety of glomerular disorders, cystatin may outperform creatinine as a prognostic marker. The ROC curve verified this, indicating that the area under the curve for Cystatin C was significantly greater in serum than creatinine. (c1)

Also, Cystatin C is not affected by inflammatory conditions, muscle mass, gender, body composition, and age [56]. Because of its capacity to remain unattached to protein, it is superior to other variables in diagnosing symptoms of renal degradation. It is also freely filtered by the glomeruli. As with other low molecular weight proteins, it is virtually fully reabsorbed into the proximal tubules [57]. The increased value of Cystatin C is also associated with an increased risk of disease cardiovascular and atherosclerotic development [58].

Regarding the positive relationship between the enzyme and age, the results of this study agreed with those of Alloush *et al.* (2011), who found a significant relationship in the enzyme activity in the group of patients with chronic renal impairment at an advanced age at a probability level of (P 0.05). Because cystatin - C is mainly located in the kidney, investigations have shown that its levels rise in cases of renal tubulopathy, indicating tissue destruction in the kidneys. This activity also increases, and thus the correlation between them is positive [70]. This is in addition to the fact that MEP has a positive association with the enzymes urea, creatinine, and uric acid, which could be related to the enzyme's activity in dissolving peptide bonds in peptides and proteins' primary substrates.

Conclusion

From the results obtained, we can conclude that the enzyme activity increases significantly in the serum of patients with DN compared with healthy patients. Moreover, this is due to damage to the tissues and cells of

the kidney. This leads to the release of the enzyme into the bloodstream and its increased activity in it because of the presence of the enzyme in abundant quantities in the tissues of the kidney. Therefore, it is recommended to adopt the enzyme as one of the essential clinical variables in diagnosing patients as an early predictor of disease.

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