Clinical Study of Homocysteine and Cysteine Levels and Some Kidney Function Parameters with Diabetic Nephropathy in thi Qar Governorate

Abeer Abbas Shaher Alzaiadi[1], Jamal Harbi Hussein Alsaadi[2]
[1, 2]Department of Chemistry College of Sciences University of Thi-Qa, Thi-Qar, 6400, Iraq
Email: abeer.abb.ch@sci.utq.edu.iq

Abstract

Diabetic nephropathy is one of the most common and serious complications of long-standing type 2 diabetes mellitus. Diabetes mellitus is a metabolic disorder characterized by hyperglycemia and disturbed metabolism of carbohydrates, proteins, and lipids. Homocysteine levels play an important role in the pathogenesis of diabetic microvascular complications, particularly diabetic nephropathy. As a result, the goal of our study is to investigate the relationship between homocysteine and cysteine in diabetic patients as well as the levels of kidney function (urea, creatinine, and uric acid) in diabetic nephropathy.The results revealed that the levels of homocysteine and cysteine in patients with renal failure (11.85±1.73 nmol/ml;577.33±81.92 µmol/l), diabetic nephropathy (8.34±1.63 nmol/ml;495.23±58.76 µmol/l), and diabetes mellitus (7.56±1.44 nmol/ml;462.47±66.61 µmol/l) were considerably higher than those in healthy controls (2.89±0.43nmol/ml;347.43±25.63µmol/l).The results demonstrated a significant increase in RBS and HbA1c levels in patients with renal failure (257.40±33.64mg/dl ;8.47±1.69),Diabetic Nephropathy (215.86±17.49 mg/dl; 9.75±2.03) and Diabetes mellitus (181.41±13.00 mg/dl ;7.51±1.47) were significantly higher than in healthy controls (85.24±5.75 mg/dl ;4.98±0.41). Where a positive association existed between homocysteine levels and RBS, HbA_{1c}.The results demonstrated a significant rise in urea, creatinine and Urate in renal failure (82.47±18.69 mg/dl ;4.42±0.87 mg/dl ;11.31±2.43 mg/dl) and Diabetic Nephropathy (72.16±11.47 mg/dl; 2.95± 0.38 mg/dl; 8.86±1.08 mg/dl) and Diabetes Mellitus patients (33.77±9.10 mg/dl ; 0.74±0.21mg/dl ; 6.49±1.83 mg/dl) were significantly higher than in healthy controls (27.18±6.10 mg/dl ; 0.58±0.06 mg/dl ;4.73±0.84 mg/dl). There was a positive association between homocysteine levels and urea, creatinine, and Urate. According to the findings of our study, serum homocysteine levels were considerably higher in-patient groups as compared to healthy groups, and homocysteine is linked to higher levels of cysteine, RBS, HbA_{1c}, urea, creatinine, and urate.

1. Introduction

Diabetes mellitus (DM) is a metabolic disturb characterized by hyperglycemia and disordered metabolism of carbohydrates, proteins and lipids (WHO, 1999; Yaribeygi et al., 2020). DM is clinically manifested by raised blood glucose level. The distinguishing symptoms of DM comprise polydipsia (excessive thirst), polyuria (excessive urine), blurring of vision and loss of weight (ADA, 2021; IDF, 2013). The chronic hyperglycemia of diabetes is related with comparatively special long- term microvascular complications (Goldenberg & Punthakee, 2013). All categories of DM are distinguish by the development of diabetes-specific microvascular complications in the eyes, kidneys and peripheral nerve system, and macrovascular complications in arteries that endow the heart, brain and nether extremities. As a result of microvascular complications, diabetes is the main cause of chronic kidney disease (CKD), blindness, and a variety of debilitating nerve pathologies. Diabetic patients are also at a lot higher risk of stroke, myocardial infarction and nether limb amputation. Though diabetic complications consist of a heterogeneous group of diseases, diabetic nephropathy is the most diffuse form (Ritz,et al, 2011). According to the rating of diabetes as proposed by the American Diabetes Association (ADA) Diabetes can be rated into the four general categories:(Type 1 diabetes mellitus, Gestational Diabetes mellitus, Type 2 diabetes mellitus , Specific Types of Diabetes (ADA, 2014). Diabetic Nephropathy (DN) is a common chronic microvascular consequence in type 2 diabetes patients, which is damage or illness of kidney. Renal failure (RF) is the inability of the kidneys to perform excretory functions, resulting in the accumulation of nitrogenous wastes in the circulation (Bikbov et al., 2020). Low urine production, swelling of the legs, ankles, and feet caused by fluid retention caused by the kidneys' failure to remove water waste, unexplained shortness of breath or tiredness, and persistent nausea are all possible signs (Breyer & Susztak, 2016). The most common cause of DN is kidney harm caused by constant hyperglycemia, which can harm the entire kidney, including the glomerulus, renal interstitium, and renal arteries. Furthermore, if T2DM patients also smoke, have hypertension, hyperlipidemia, or other risk factors, they are more likely to develop DN. (Ahmed et al., 2013; Al-Rubeaan et al., 2014; Unnikrishnan et al., 2007). Diabetic renal disease has a natural history that

doi.org/10.31838/hiv23.01.109 Received: 11.09.22, Revised: 22.10.22, Accepted: 04.12.22

includes glomerular hyperfiltration, increased albumin in urine, a decrease in Glomerular filtration rate (GFR), and finally end stage renal disease. (Alicic et al., 2017). Diabetic nephropathy is clinically distinguished by a progressive rise in proteinuria and decrease in GFR, hypertension, and an increased risk of cardiovascular morbidity and mortality (Ayodele et al., 2004). Homocysteine (Hcy) is an amino acid involve a sulfhydryl group that is produced when methionine is demethylated and is required for intravascular metabolism. Homocysteine is known to be a risk factor for atherosclerotic vascular disease processes that contribute to cardiovascular disease (CVD)(Sanchez-Rodriguez et al., 2020). It has recently been revealed that serum Hcy levels are related to the degree of renal function harm (Xu et al., 2019). With evolution of renal damage, serum Hcy was also rose correspondingly (Ma et al., 2020). Elevated total plasma homocysteine tHcy is venomous to cells and is linked to a various health problems (Hannibal & Blom, 2017). Cysteine (Cys) the primary sulfur-containing amino acid (SAA) is a semi essential amino acid (AA) because it can be obtained from the diet or produced from methionine degradation via the transsulfuration pathway. In the mammalian diet, cysteine is considered as representative of SAAs (Bin et al., 2017). Within the body, cys is synthesized in the liver from Hcy by transmethylation of methionine. First, Hcy is condensed with serine by CBS and then cleavage of CBS produces cysteine. During TSS, serine gives its carbon chain to cysteine and sulfur atom of cys comes from methionine. Within the body, cys catabolic pathways are sources of the synthesis of coenzyme A, glutathione, taurine, and oxidized and reduced inorganic sulfur. In the liver, two catabolic pathways of cysteine take place which includes oxidative pathway and desulfuration pathway, respectively. Briefly, in the oxidative pathway, cysteine sulfinate (intermediate in cysteine metabolism) is either transaminated to produce sulfite and pyruvate or decarboxylated to form taurine. The desulfuration pathway ends up with hydrogen sulfide and pyruvate. If the supply of cysteine is high, then the oxidative pathway is superior over desulfuration pathway and the desulfuration pathway increases when cysteine supply is low (Papet et al., 2019).

Metabolism of Hcy involves two pathways, which mainly include re-methylation and trans-sulphuration. Re-methylation is the process that requires methyl group for the conversion of Hcy into methionine, and the Re-methylation process is carried out by BHMT in the kidney and liver.Transculturation involves attachment of Hcy with serine and formation of cystathionine (a sulfur metabolite produced from Hcy) with the help of CBS (an enzyme) and vitamin B6 which acts as a coenzyme to synthesize cysteine (Hannibal & Blom, 2017; Ntaios, 2015).

2. Materials and Method

The study was carried out on 149 women and man had attended at Nasiriyah Teaching Hospital, Al-Hussein Teaching Hospital, Al-Souq Al-Shuyoukh Hospital, and specialized clinics. during the period between December (2021) to March (2022).149 cases divided in to four groups:

The control group ranging age (30-80) years, a group of kidney failure ranging age (30-80) years, a group of diabetes ranging age (30-80) years, and a group of diabetic nephropathy ranging age (41-80) years.

The homocysteine and Cysteine were measured using the ELISA technique (BT LAB – China;MyBioSource), And all of the Urea, Creatinine and Uric Acid were measured using Copas COBAS INTEGRA systems (Ferguson et al., 1964). Collection of blood Samples:

Venous blood (5 ml) was collected from patients and healthy then divided into two parts, part one It was 1 mL and was added to EDTA containing – Ethylene diamine tetra acetic acid (EDTA) plasma, and gently shaken for use in determining for HbA_1c estimation. The second part (4 ml) was transferred to a normal tube without the anticoagulant (serum) which recognized the clotting for 20 minutes at room temperature. After the blood clot, it was transferred to a centrifuge at (3000) xg in order to obtain the serum. The collected serum is used to estimate the variables in this study as it was stored in the freeze at (-80 ° C) until use. The test is based on the ELISA principle (Enzyme linked Immunosorbent Assay). The used reagents were supplied by Bioassay Technology Laboratory and MyBioSource.

3. Statistical Analysis

Statistical analysis was based on one way ANOVA (analysis of variations test with LSD (least significant difference), differences were considered to be significant if $p < 0.05$. Statistical analysis was carried out using SPSS statistical version 23.0 SPSS Inc, Chicago, 111.

4. Results and Discussion

Clinical and Characteristic Features of the Study Groups:

In the present study, a total of 149 subjects participated; 116 patients were divided into three groups (DM, DN, RF) and compared to a control group of healthy subjects (33). Tabulated characteristics for all studied groups are presented in Table 1.

Serum Homocysteine and cysteine Concentration

Figure (2) and table (1) Show that the levels of homocysteine in the patient groups are higher than the levels in the control groups ($p<0.05$). It was found that the levels of homocysteine were much higher in the group with renal failure than in the groups with diabetic nephropathy and diabetes mellitus ($p<0.05$). As renal function gets worse, Hcy levels rise, and patients become less responsive to the usual treatments for lowering Hcy. The healthy kidney seems to play a big role in the clearance and metabolism of Hcy, as it does with other amino acids. (Friedman et al., 2001). Additionally, a significant rise

in homocysteine levels was observed in the group of people with diabetic nephropathy when compared to those with diabetes mellitus ($p< 0.05$). According to our study, serum homocysteine levels in DN and Type 2 DM patients were 8.341.63 and 7.561.44, respectively. This finding suggested that DN patients' serum Hcy levels were higher than those of Type 2 DM patients' and that this difference was associated with patients' renal damage. This outcome coincided with those of a study of (Thipsawat, 2021).

Table 1: Characteristic data for studied groups				
(M/F)	BMI(Kg/m2) Mean ±SD	Age (years) Mean ±SD	NO.	Groups
20/17	27.51±5.4	59.14±14	37	Renal Failure
22/14	27.33±5.75	64±14	36	Diabetic Nephropathy
18/25	28.71±3.58	49±14	43	Diabetes Mellitus
19/14	26.14±6.32	41±15	33	Control
No: Number of subjects.SD: Standard deviation. **LSD:** Least Significant Difference (a, b, c) Means having different letters in the same column differed significantly (P≤0.05).				

Table 2: Serum homocysteine and cysteine levels of controls and patients groups			
Cysteine(µmol/L) Mean ±SD	Homocysteine(nmol/mL) Mean ±SD	NO.	Groups
577.33±81.92 a	11.85±1.73 a	37	Renal Failure
495.23±58.76 b	8.34±1.63 b	36	Diabetic Nephropathy
462.47±66.61 b	7.56±1.44 c	43	Diabetes Mellitus
347.43±25.63 d	2.89±0.43 d	33	Control
41.64	0.50		LSD

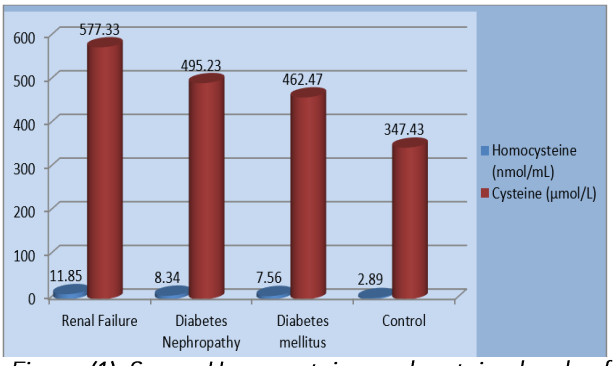

Figure (1): Serum Homocysteine and cysteine levels of control and patient Groups

According to table (2) and the same figure (1), cysteine levels in the patient groups were significantly higher than in the control groups ($p< 0.05$). Cysteine levels were found to be significantly higher in the group with renal failure when compared to the groups with diabetic nephropathy and diabetes mellitus ($p< 0.05$). In addition, cysteine levels were found to be similar in the diabetic nephropathy and diabetes mellitus groups. This result coincided with the results of a study (Valente *et al.*, 2012;Pastore *et al.*, 2015).

Figure (2) shows positive correlation between serum homocysteine and cysteine in RF group with correlation coefficient (r= 0.11) , positive correlation in DN group with correlation coefficient (r= 0.12) and positive correlation in DM group with correlation coefficient (r= 0.15)

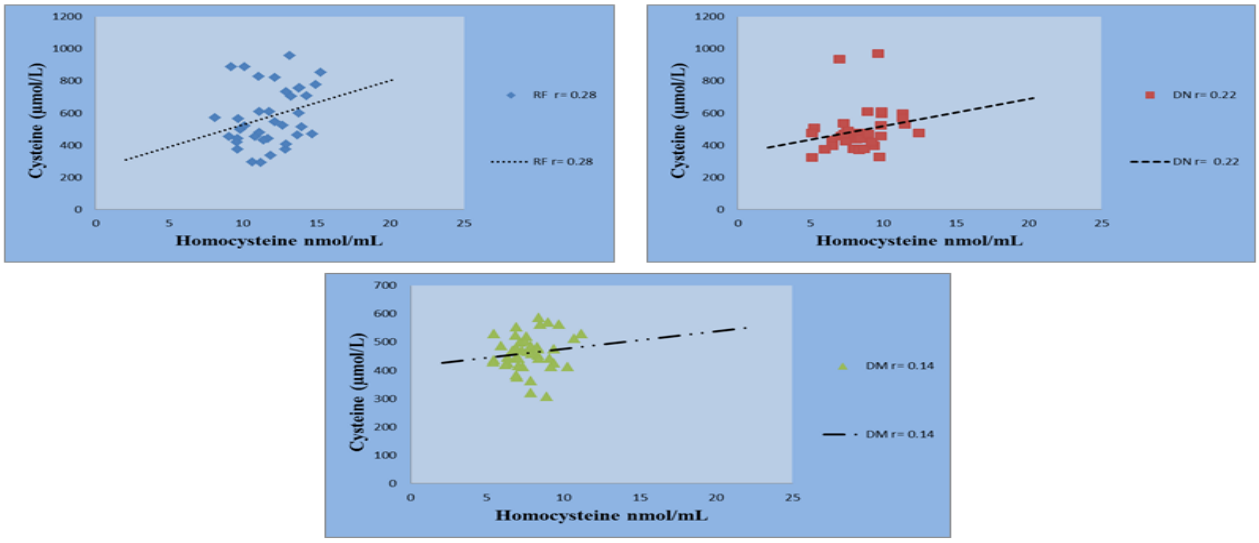

Figure (2): correlation between serum homocysteine and cysteine in patient Groups

Random Blood Sugar Concentration

Table (3) and figure (3) Shaw a significant increase in the levels of RBS in patients' groups in comparision with controls group ($p<0.05$). It was found a significant increase in the levels of RBS in renal failure group in comparision with (diabetic nephropathy and diabetes mellitus) groups ($p<0.05$). Also, it was found a significant increase in the levels of RBS in diabetic Nephropathy group in comparision with diabetes mellitus group ($p<0.05$).

Table (3): Serum Random Blood Sugar levels of control and patients groups		
RBS (mg/dL) Mean ±SD	NO.	Groups
257.40±33.64 a	37	Renal Failure
215.86±17.49 b	36	Diabetic Nephropathy
181.41±13.00 c	43	Diabetes Mellitus
85.24±5.75 d	33	Control
7.25		LSD

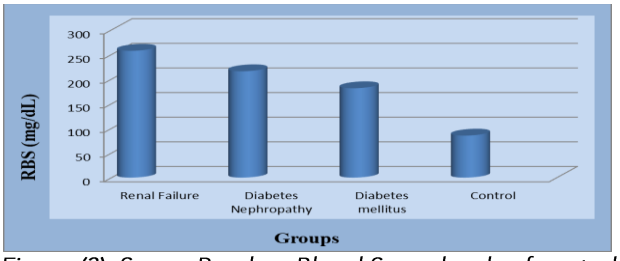

Figure (3): Serum Random Blood Sugar levels of control and patient

Our study demonstrates that poorly managed blood sugar levels would raise serum urea levels, increasing the likelihood that the patient would develop diabetic nephropathy. This is consistent with other studies' findings that hyperglycemia is one of the main factors contributing to progressive renal damage (Anjaneyulu & Chopra, 2004; Bamanikar *et al.*, 2016; Shrestha *et al.*, 2008).

One of the main causes of chronic renal failure is diabetic nephropathy, which can be prevented by maintaining good blood glucose control (Bamanikar *et al.*, 2016).

Figure (4) shows positive correlation between serum homocysteine and RBS in RF group with correlation coefficient (r= 0.13), positive correlation in DN group with correlation coefficient (r= 0.14) and positive correlation in DM group with correlation coefficient (r= 0.22).

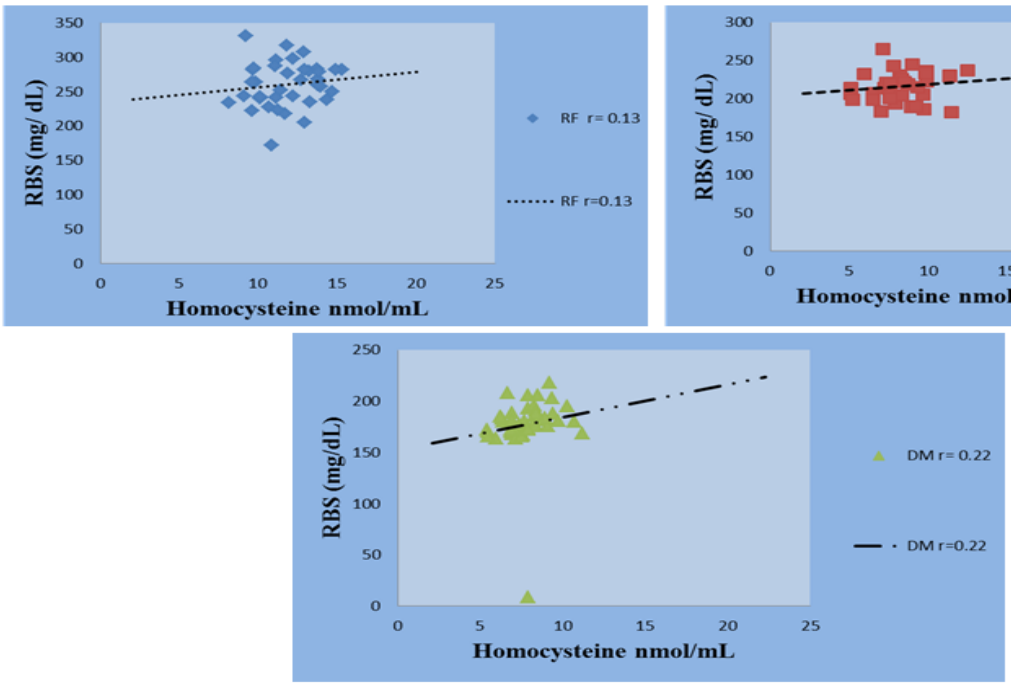

Figure (4): Correlation between serum Hcy and Random Blood Sugar in patients' group and control group.

Hemoglobin A_1c Ratio

Table 4 and Figure 5 show a statistically significant rise in HbA_1c levels in the patient groups when compared to the control groups ($p < 0.05$). HbA_1c levels were found to be significantly higher in the diabetic nephropathy group when compared to the groups with diabetes mellitus and renal failure ($p < 0.05$). Additionally, a statistically significant rise in HbA_1c levels was discovered in the group suffering from renal failure when compared to the diabetes mellitus group ($p < 0.05$).

Table (4): Serum Hemoglobin A1c levels of control and patients' groups		
HbA_1c (%) Mean ±SD	NO.	Groups
8.47±1.69 b	37	Renal Failure
9.75±2.03 a	36	Diabetic Nephropathy
7.51±1.47 c	43	Diabetes Mellitus
4.98±0.41 d	33	Control
0.54		LSD

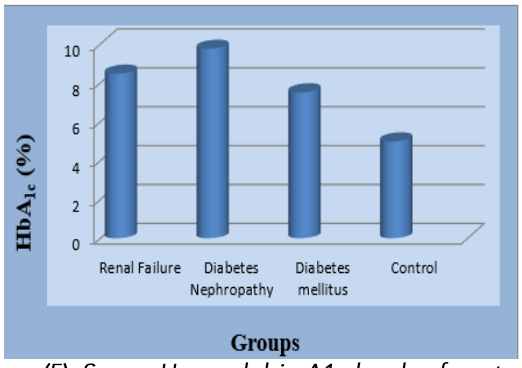

Figure (5): Serum Hemoglobin A1c levels of control and patient groups

The results of Essam El-Din's study are the same as the results of our study in high HbA_1c in diabetic nephropathy compared to diabetes.(M. Esam El-Din *et al.*, 2022). The HbA_1c concentration can predict diabetes complications because it shows the more harmful glycation effects of diabetes, like retinopathy and nephropathy, which are thought to be caused by

detrimental advanced glycation end products (Castilho *et al.*, 2003).

These findings demonstrate that DN patients had higher HbA_1c levels, which increase the risk of developing micro vascular problems due to poor glycemic management. Furthermore, as the disease's duration increases, glycemic control gradually deteriorates. These findings concur with those of a research by Shestakova MV(Shestakova *et al.*, 1993). Diabetic nephropathy is more common in type 2 diabetes patients with poor glycemic control. (Mogensen, 2004) In the present study, type 2 DM patients with nephropathy have poor glycemic control compared to those without nephropathy, as evidenced by higher levels of HbA_1c in the DN group. This result is also supported by studies showing that improving glycemic control reduces the risk of nephropathy development in type 2 DM. (Group, 1998; Mulec *et al.*, 1998; Nathan *et al.*, 1993).

Figure (6) shows positive correlation between serum homocysteine and HbA_1c in RF group with correlation coefficient (r= 0.14), positive correlation in DN group with correlation coefficient (r= 0.20) and positive correlation in DM group with correlation coefficient (r= 0.30)

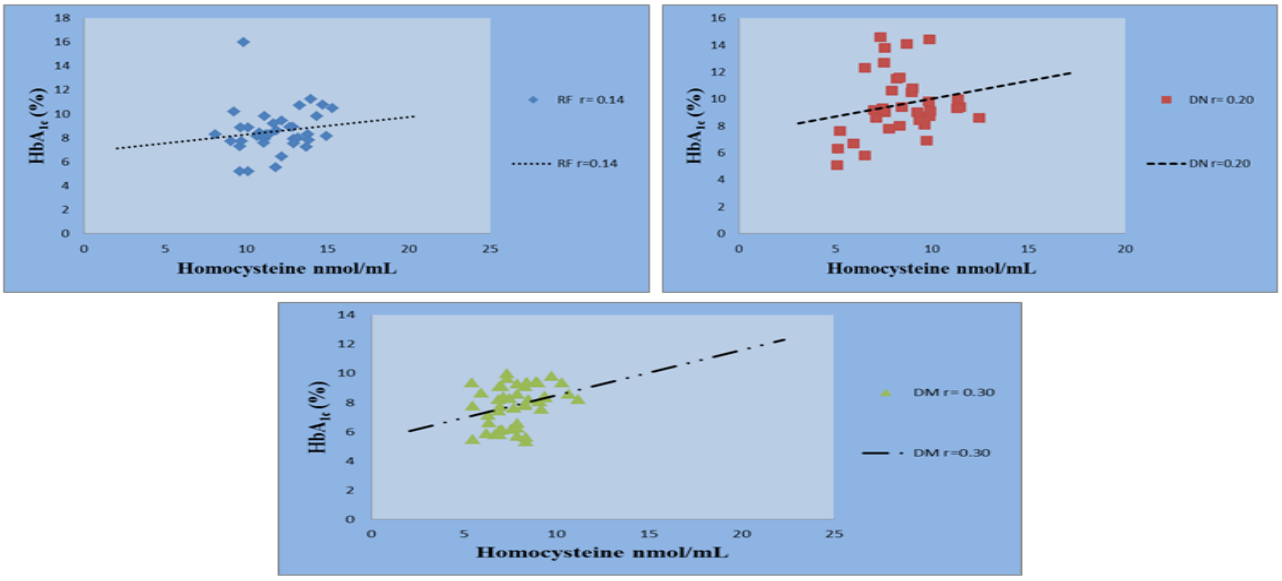

Figure (6): Correlation between serum Hcy and Hemoglobin A_1c in patients' groups and control groups

Serum Urea Concentration

Table (5) and figure (7) Shaw a significant increase in the levels of urea in patient groups in comparison with the control group ($p<0.05$). There was a significant increase in the levels of urea in the renal failure group in comparison with the (diabetic nephropathy and diabetes mellitus) groups ($p<0.05$). Also, it was found to be a significant increase in the levels of urea in the diabetic nephropathy group in comparison with the diabetes mellitus group ($p<0.05$). This result matched with the results of the study (AbuMustafa & Yassin, 2017; Al-Fartosy *et al.*, 2021).

Table (5): Serum Urea and Creatinine levels of controls and patients groups			
Creatinine (mg/dL) Mean ±SD	Urea (mg/dL) Mean ±SD	NO.	Groups
4.42±0.87 [a]	82.47±18.69 [a]	37	Renal Failure
2.95± 0.38 [b]	72.16±11.47 [b]	36	Diabetic Nephropathy
0.74±0.21 [c]	33.77±9.10 [c]	43	Diabetes Mellitus
0.58±0.06 [d]	27.18±6.10 [d]	33	Control
0.18	4.33		LSD

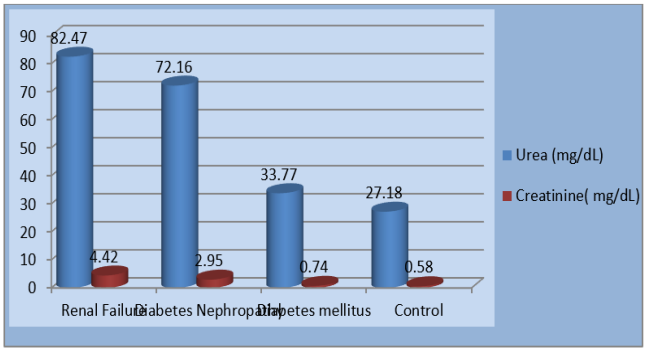

Figure (7): Serum urea and creatinine levels of controls and patients' groups

In patients with CKD, high serum levels of urea, creatinine, and uric acid were the disease's hallmark symptoms (Zhang et al., 2019). This study found that CKD patients had considerably higher Hcy levels than the healthy control group. One important location for HCy metabolism is the kidney. HCY produced by the body can either be remethylated into methionine or transsulfurated into cysteine. Renal epithelial cells use the re-methylation and transsulfuration pathways. However, it seems that the proximal tubule's epithelial cells mostly degrade HCY through transsulfuration. Hyperhomocysteinemia is caused by an increase in plasma and tissue HCY levels due to poor clearance. For people with CKD, hyperhomocysteinemia poses

a concern. Vasoconstriction and renal microvasculature dysfunction are caused by HCY buildup. A vicious loop results from the decline in renal function because more HCY builds up as a result, causing chronic renal failure (Yang et al., 2016). The significant prevalence of hyperhomocysteinemia in CKD patients has stoked speculation about hyperhomocysteinemia's potential function as a risk factor for the disease's progression (Marti *et al.*, 2011; Ponte *et al.*, 2013). In accordance with findings from another investigation, our data showed that urea levels were higher in T2DM patients with and without DN when compared to healthy controls (Adnan *et al.*, 2020). The results of our study were similar to those of other studies, which showed that high plasma urea levels in people with diabetes may be a sign of a problem before the kidneys (Adler *et al.*, 2003; Bamanikar *et al.*, 2016; Judykay, 2007).

Figure (8) shows positive correlation between serum homocysteine and urea in RF group with correlation coefficient (r= 0.15) , positive correlation in DN group with correlation coefficient (r= 0.27) and positive correlation in DM group with correlation coefficient (r= 0.18).Serum homocysteine level were significantly higher in CKD patients compared to controls ,Homocysteine levels were positively correlated with urea (Nada, 2012).

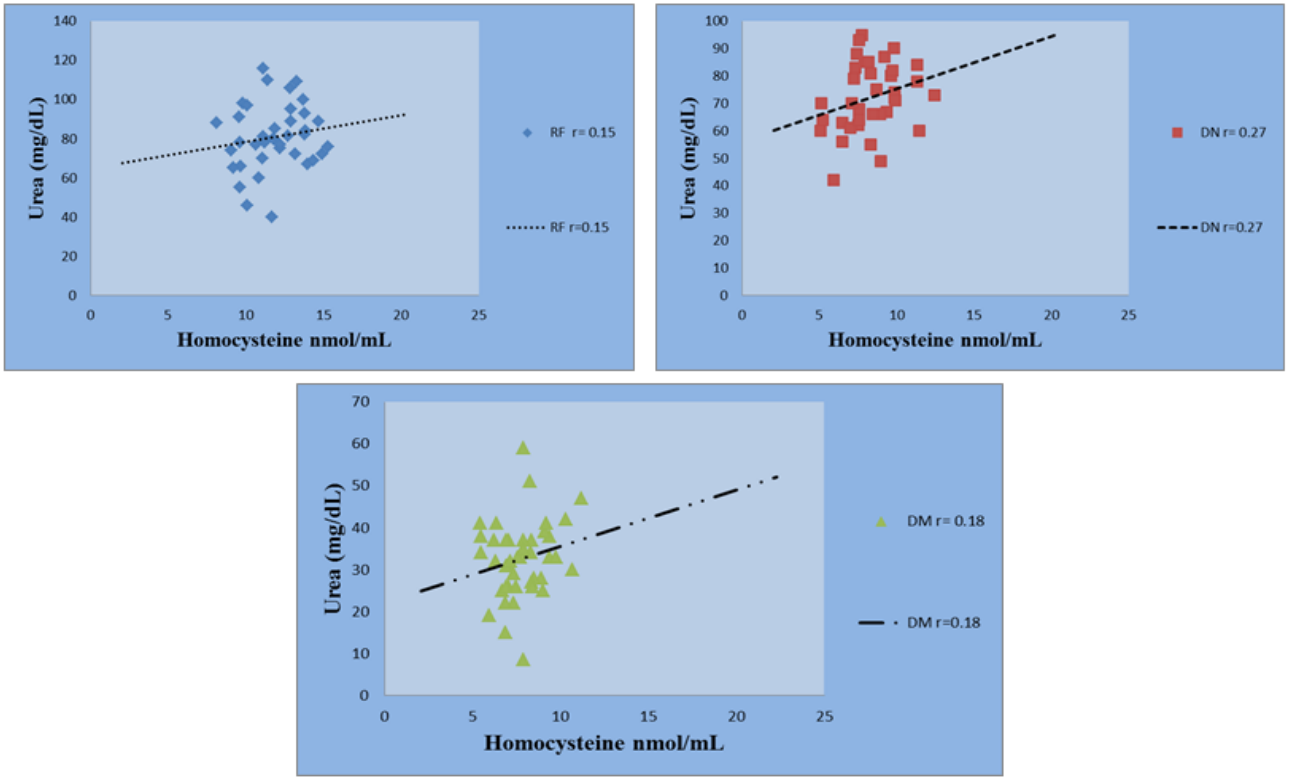

Figure (8): Correlation between serum Hcy and urea in patients' groups and controls groups

Serum Creatinine Concentration

Table (5) and Figure (7) explain a statistically significant rise in creatinine levels in the patient groups when compared to the control groups (p< 0.05). In comparison to the groups with diabetic nephropathy and diabetes mellitus, it was discovered that the levels of creatinine were significantly higher in the renal failure group (p< 0.05). Additionally, it was discovered that the levels of creatinine were significantly higher in the group with diabetic nephropathy than in the group with diabetes mellitus (p< 0.05). This result matched those of a study(AbuMustafa & Yassin, 2017; Al-Fartosy *et al.*, 2021). When the decline of renal function rises 50%, the level of serum creatinine often increases (Lee *et al.*, 2013). When compared to healthy controls, our data showed that creatinine levels were higher in T2DM patients with and without DN. This finding is consistent with that from another study that found the same results(Adnan *et al.*, 2020). In comparison to serum urea level, serum creatinine is a more sensitive indicator of renal function. This is due to the fact that creatinine mostly satisfies the criteria for a perfect filtration marker (Adler *et al.*, 2003; Deepa *et al.*, 2011). If a microalbuminuria screening test cannot be conducted, blood urea and creatinine are simple indicators accessible in individuals with proteinuria to prevent the progression of diabetes mellitus to diabetic nephropathy. We would like to conclude that blood urea and serum creatinine levels are useful and easy assays for assessing renal function in poorly managed diabetes.(Bamanikar *et al.*, 2016). Figure (9) depicts a positive correlation between serum homocysteine and creatinine in the RF group with a correlation coefficient of (r = 0.20), a positive correlation in the DN group with a correlation coefficient of (r = 0.13), and a positive correlation in the DM group with a correlation coefficient of (r = 0.14). The levels of homocysteine in the blood of CKD patients were much higher than those of the controls, and the levels of homocysteine were linked to the levels of creatinine (Nada, 2012).

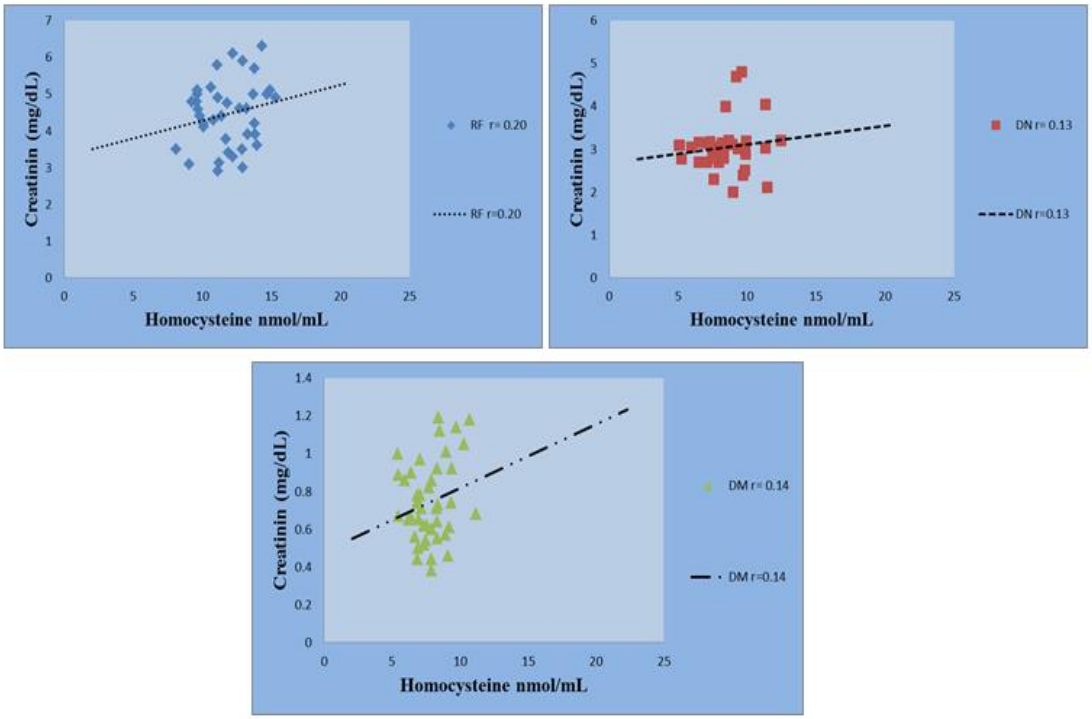

Figure (9): Correlation between serum Hcy and creatinine in patients' group and control group

3.2.6. surem Uric Acid Concentration

Table (6) and figure (10) demonstrate a statistically significant rise in UA values in patient groups compared to controls ($p < 0.05$). In comparison to the groups with diabetic nephropathy and diabetes mellitus, it was discovered that the levels of UA were significantly higher in the renal failure group ($p < 0.05$). Also, it was found that the levels of UA in the group with diabetic nephropathy were significantly higher than in the group with diabetes mellitus ($p < 0.05$).

Table (6): Serum Uric Acid levels of control and patient groups

Uric Acid (mg/dL) Mean ±SD	NO.	Groups
11.31±2.43 a	37	Renal Failure
8.86±1.08 b	36	Diabetic Nephropathy
6.49±1.83 c	43	Diabetes Mellitus
4.73±0.84 d	33	Control
0.59		LSD

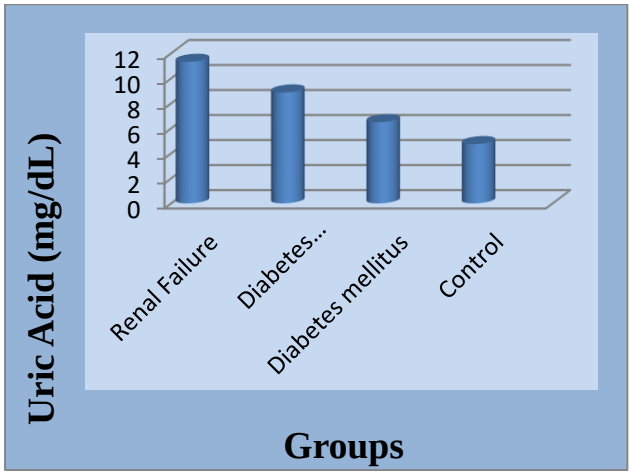

Figure (10): Serum Uric Acid levels of controls and patient groups

The amount of Hcy can be determined from SUA levels in the body.Uric acid and Hcy are both excreted by the kidneys at the same time. Hcy will pilling up in the body if the level of eGFR decrease, and this pilling up of Hcy will cause kidney damage and additional eGFR decline (Omer Sultan et al., 2020; Wang et al., 2015; Yi, 2007). Because of the endothelial dysfunction brought on by SUA, oxidative stress is rised, which leads to microvascular illnesses that can encourage the growth of vascular smooth muscle cells and decline the bioavailability of endothelial nitric oxide (Kanbay et al., 2013). According to related studies, an elevated SUA level is determined as 5.5 mg/dL. (Feig & Johnson, 2003; Loeffler et al., 2012). The following is a proposed mechanism underlying the link between uric acid and Hcy: Methoinine can be transformed into S-adenosylhomocysteine (SAH), which can subsequently be turned into Hcy and adenosine in the human body (Palmer & Abeles, 1979), Hcy can regenerate methyl methoinine (De La Haba & Cantoni, 1959), and adenosine can be metabolized into urate. (Waring et al., 2000).

According to Figure (11), there is a positive association between serum homocysteine and uric acid in the RF group with a correlation coefficient of ($r=0.14$), in the DN group with a correlation coefficient of($r= 0.15$), and in the DM group with a correlation coefficient of($r= 0.25$). In CKD patients, serum homocysteine levels were considerably higher than in healthy controls, and uric acid levels and homocysteine levels were positively associated (Nada, 2012). In these CKD patients, uric acid and plasma homocysteine levels were related. High serum homocysteine levels were linked to patients' having decreased renal function, and HHcy was prevalent in Chinese patients with CKD. (Ye et al., 2016).

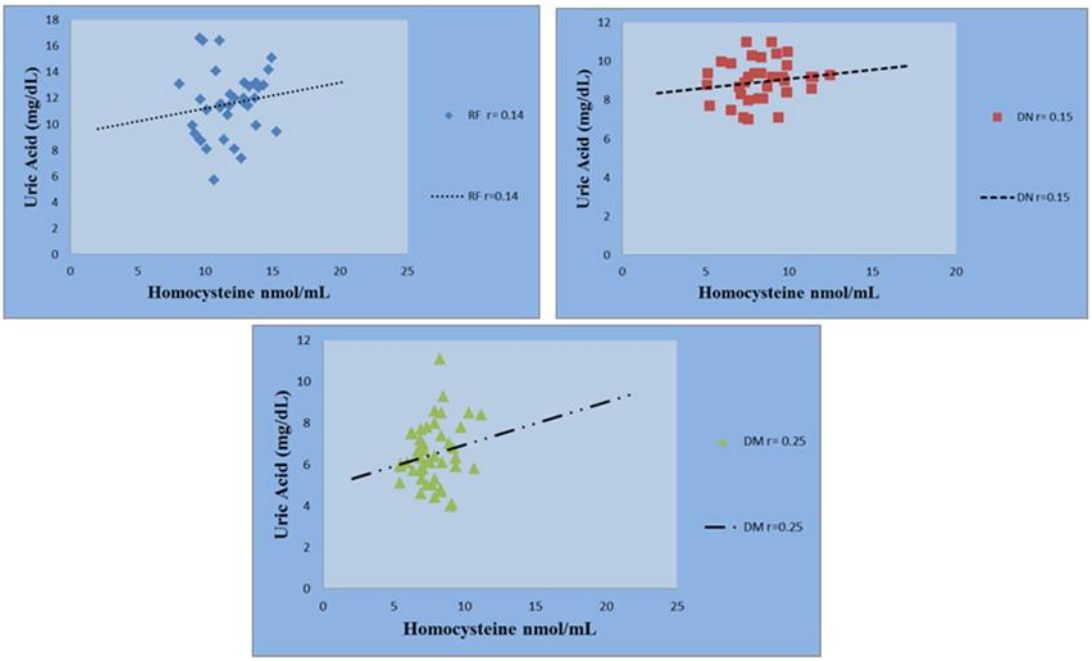

Figure (11): Correlation between serum Hcy and Uric Acid in patients' groups and controls groups

References

AbuMustafa, A. M., & Yassin, M. M. (2017). Clinical and biochemical aspects associated with diabetic nephropathy among type 2 diabetic males in Gaza strip. *Int J Med*, *5*.

Adler, A. I., Stevens, R. J., Manley, S. E., Bilous, R. W., Cull, C. A., & Holman, R. R. (2003). Development and progression of nephropathy in type 2 diabetes: The United Kingdom Prospective Diabetes Study (UKPDS 64). *Kidney International*, *63*(1), 225–232. https://doi.org/10.1046/j.1523-1755.2003.00712.x

Adnan, J. M. A.-F., Nadhum, A. A., & Sadoun, A. A. (2020). Insulin resistance and specific biomarkers in blood and urine of type 2 diabetic patients with or without nephropathy in Basrah, Iraq. *African Journal of Biochemistry Research*, *14*(4), 125–134.

Ahmed, M. A., Kishore, G., Khader, H. A., & Kasturirangan, M. N. (2013). Risk factors and management of diabetic nephropathy. *Saudi Journal of Kidney Diseases and Transplantation*, *24*(6), 1242.

Al-Fartosy, A. J. M., Awad, N. A., & Alsalimi, S. A. (2021). Clinical markers and some trace elements in patients with type-2 diabetic nephropathy: Impact of insulin resistance. *The Journal of Medical Investigation*, *68*(1.2), 76–84.

Al-Rubeaan, K., Youssef, A. M., Subhani, S. N., Ahmad, N. A., Al-Sharqawi, A. H., Al-Mutlaq, H. M., David, S. K., & AlNaqeb, D. (2014). Diabetic nephropathy and its risk factors in a society with a type 2 diabetes epidemic: a Saudi National Diabetes Registry-based study. *PloS One*, *9*(2), e88956.

Alicic, R. Z., Rooney, M. T., & Tuttle, K. R. (2017). Diabetic kidney disease: challenges, progress, and possibilities. *Clinical Journal of the American Society of Nephrology*, *12*(12), 2032–2045.

American Diabetes Association. (2014). Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care*, *37*, 81–90.

American Diabetes Association. (2021). 2. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes—2021. *Diabetes Care*, *44*(Supplement_1), S15–S33. https://doi.org/10.2337/dc21-S002

Anjaneyulu, M., & Chopra, K. (2004). Quercetin, an anti-oxidant bioflavonoid, attenuates diabetic nephropathy in rats. *Clinical and Experimental Pharmacology and Physiology*, *31*(4), 244–248.

Ayodele, O. E., Alebiosu, C. O., & Salako, B. L. (2004). Diabetic nephropathy--a review of the natural history, burden, risk factors and treatment. *Journal of the National Medical Association*, *96*(11), 1445.

Bamanikar, S. A., Bamanikar, A. A., & Arora, A. (2016). Study of Serum urea and Creatinine in Diabetic and nondiabetic patients in a tertiary teaching hospital. *The Journal of Medical Research*, *2*(1), 12–15.

Bikbov, B., Purcell, C. A., Levey, A. S., Smith, M., Abdoli, A., Abebe, M., Adebayo, O. M., Afarideh, M., Agarwal, S. K., & Agudelo-Botero, M. (2020). Global, regional, and national burden of chronic kidney disease, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. *The Lancet*, *395*(10225), 709–733.

Bin, P., Huang, R., & Zhou, X. (2017). Oxidation Resistance of the Sulfur Amino Acids: Methionine and Cysteine. *BioMed Research International*, *2017*, 1–6. https://doi.org/10.1155/2017/9584932

Breyer, M. D., & Susztak, K. (2016). The next generation of therapeutics for chronic kidney disease. *Nature Reviews Drug Discovery*, *15*(8), 568–588. https://doi.org/10.1038/nrd.2016.67

Castilho, E. M., Glass, M. L., & Manço, J. C. (2003). The effects of 2, 3-diphosphoglycerate, adenosine triphosphate, and glycosylated hemoglobin on the hemoglobin-oxygen affinity of diabetic patients. *Brazilian Journal of Medical and Biological Research*, *36*, 731–737.

De La Haba, G., & Cantoni, G. L. (1959). The enzymatic synthesis of S-adenosyl-L-homocysteine from adenosine and homocysteine. *Journal of Biological Chemistry*, *234*(3), 603–608.

Deepa, K., B.K, M. goud, Devi, O. S., R.N, D., Nayal, B., Prabhu, A., & Anwar, N. (2011). Serum Urea, Creatinine In Relation To Fasting Plasma Glucose Levels In Type 2 Diabetic Patients. *International Journal of Pharmacy and Biological Sciences*, *1*(3), 279–283.

Esam El-Din Mostafa Amer, Y., Khalil Ahmed Esa, Y., El-Adl El-Adl, H., & Kidr, A. E.-H. (2022). STUDY OF SERUM FERRITIN AND DIABETIC NEPHROPATHY IN TYPE 2 DIABETES IN EGYPTIANS. *Al-Azhar Medical Journal*, *51*(3), 1637–1654.

Feig, D. I., & Johnson, R. J. (2003). Hyperuricemia in childhood primary hypertension. *Hypertension*, *42*(3), 247–252.

Friedman, A. N., Bostom, A. G., Selhub, J., Levey, A. S., & Rosenberg, I. H. (2001). The kidney and homocysteine metabolism. *Journal of the American Society of Nephrology*, *12*(10), 2181–2189.

Goldenberg, R., & Punthakee, Z. (2013). Definition, Classification and Diagnosis of Diabetes, Prediabetes and Metabolic Syndrome. *Canadian Journal of Diabetes*, *37*, S8–S11. https://doi.org/10.1016/j.jcjd.2013.01.011

Group, U. K. P. D. S. (UKPDS). (1998). Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). *The Lancet*, *352*(9131), 837–853.

Hannibal, L., & Blom, H. J. (2017). Homocysteine and disease: causal associations or epiphenomenons? *Molecular Aspects of Medicine*, *53*, 36–42.

International Diabetes Federation. (2013). IDF Diabetes Atlas, 6th edn. Brussels, Belgium: *International Diabetes Federation*.

Judykay, T. (2007). Nutrition for reducing urea and creatinine in the blood. *Diabetes Care*, *27*, 2191–2192.

Kanbay, M., Segal, M., Afsar, B., Kang, D.-H., Rodriguez-Iturbe, B., & Johnson, R. J. (2013). The role of uric acid in the pathogenesis of human cardiovascular disease. *Heart*, *99*(11), 759–766. https://doi.org/10.1136/heartjnl-2012-302535

Lee, E. Y., Lee, Y.-M., Choi, K. H., Lee, H. C., Lee, B.-W., & Kim, B. S. (2013). Comparison of two creatinine-based equations for predicting decline in renal function in type 2 diabetic patients with nephropathy in a Korean population. *International Journal of Endocrinology*, *2013*.

Loeffler, L. F., Navas-Acien, A., Brady, T. M., Miller III, E. R., & Fadrowski, J. J. (2012). Uric acid level and elevated blood pressure in US adolescents: National Health and Nutrition Examination Survey, 1999–2006. *Hypertension*, *59*(4), 811–817.

Ma, N., Xu, N., Yin, D., Liu, W., Wu, M., & Cheng, X. (2020). Relationship between plasma total homocysteine and the severity of renal function in Chinese patients with type 2 diabetes mellitus aged≥ 75 years. *Medicine*, *99*(27).

Marti, F., Vollenweider, P., Marques-Vidal, P.-M., Mooser, V., Waeber, G., Paccaud, F., & Bochud, M. (2011). Hyperhomocysteinemia is independently associated with albuminuria in the population-based CoLaus study. *BMC Public Health*, *11*(1), 1–9.

Mogensen, C. E. (2004). Microalbuminuria, blood pressure and diabetic renal disease: origin and development of ideas. *The Kidney and Hypertension in Diabetes Mellitus*, 905–960.

Mulec, H., Blohmé, G., Grände, B., & Björck, S. (1998). The effect of metabolic control on rate of decline in renal function in insulin-dependent diabetes mellitus with overt diabetic nephropathy. *Nephrology, Dialysis, Transplantation: Official Publication of the European Dialysis and Transplant Association-European Renal Association*, *13*(3), 651–655.

Nada, A. A. A. (2012). *Homocysteine levels in chronic kidney disease patients in Gaza Governorate, Gaza Strip*.

Nathan, D. M., Genuth, S., Lachin, J., Cleary, P., Crofford, O., Davis, M., Rand, L., & Siebert, C. (1993). The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *The New England Journal of Medicine*, *329*(14), 977–986.

Ntaios, G. (2015). Homocysteine, B vitamins, and cardiovascular risk. In *Foods and dietary supplements in the prevention and treatment of disease in older adults* (pp. 309–318). Elsevier.

Omer Sultan, M., Farooque, U., Javed, R., Khan, M. I., Karimi, S., Abdul Sattar, R., & Cheema, O. (2020). Correlation of Homocysteine Level and Age in Patients with Ischemic Stroke. *Cureus*. https://doi.org/10.7759/cureus.7785

Palmer, J. L., & Abeles, R. H. (1979). The mechanism of action of S-adenosylhomocysteinase. *Journal of Biological Chemistry*, *254*(4), 1217–1226.

Papet, I., Rémond, D., Dardevet, D., Mosoni, L., Polakof, S., Peyron, M.-A., & Savary-Auzeloux, I. (2019). Sulfur amino acids and skeletal muscle. In *Nutrition and skeletal muscle* (pp. 335–363). Elsevier.

Pastore, A., Noce, A., Di Giovamberardino, G., De Stefano, A., Callà, C., Zenobi, R., Dessì, M., & Di Daniele, N. (2015). Homocysteine, cysteine, folate and vitamin B12 status in type 2 diabetic patients with chronic kidney disease. *Journal of Nephrology*, *28*(5), 571–576. https://doi.org/10.1007/s40620-014-0126-4

Ponte, B., Pruijm, M., Marques-Vidal, P., Martin, P.-Y., Burnier, M., Paccaud, F., Waeber, G., Vollenweider, P., & Bochud, M. (2013). Determinants and burden of chronic kidney disease in the population-based CoLaus study: a cross-sectional analysis. *Nephrology Dialysis Transplantation*, *28*(9), 2329–2339.

Ritz, E., Zeng, X.X., Rychlik, I. (2011). Clinical manifestation and natural history of diabetic nephropathy. In *Contributions to nephrology* (pp. 170,19-27).

Sanchez-Rodriguez, E., Egea-Zorrilla, A., Plaza-Díaz,

J., Aragón-Vela, J., Muñoz-Quezada, S., Tercedor-Sánchez, L., & Abadia-Molina, F. (2020). The gut microbiota and its implication in the development of atherosclerosis and related cardiovascular diseases. *Nutrients*, *12*(3), 605.

Shestakova, M. V, Dedov, I. I., Neverov, N. I., Severgina, E. S., Diuzhev, T. G., & Ponomarev, A. B. (1993). Hyperlipidemia as a factor in the development and progress of diabetic nephropathy. *Problemy Endokrinologii*, *39*(5), 7–9.

Shrestha, S., Gyawali, P., Shrestha, R., Poudel, B., & Sigdel, M. (2008). Serum urea and creatinine in diabetic and non-diabetic subjects. *Journal of Nepal Association for Medical Laboratory Sciences P*, *9*(1), 11–12.

Thipsawat, S. (2021). Early detection of diabetic nephropathy in patient with type 2 diabetes mellitus: A review of the literature. *Diabetes and Vascular Disease Research*, *18*(6), 14791641211058856.

Unnikrishnan, R., Rema, M., Pradeepa, R., Deepa, M., Shanthirani, C. S., Deepa, R., & Mohan, V. (2007). Prevalence and risk factors of diabetic nephropathy in an urban South Indian population: the Chennai Urban Rural Epidemiology Study (CURES 45). *Diabetes Care*, *30*(8), 2019–2024.

Valente, A., Bronze, M. R., Bicho, M., Duarte, R., & Costa, H. S. (2012). Validation and clinical application of an UHPLC method for simultaneous analysis of total homocysteine and cysteine in human plasma. *Journal of Separation Science*, *35*(24), 3427–3433. https://doi.org/10.1002/jssc.201200672

Wang, H., Cui, K., Xu, K., & Xu, S. (2015). Association between plasma homocysteine and progression of early nephropathy in type 2 diabetic patients. *International Journal of Clinical and Experimental Medicine*, *8*(7), 11174.

Waring, W. S., Webb, D. J., & Maxwell, S. R. J. (2000). Uric acid as a risk factor for cardiovascular disease. *Qjm*, *93*(11), 707–713.

WHO. (1999). *Definition, diagnosis and classification of diabetes mellitus and its complications*. World health organization.

Xu, W., Tang, S., Xiang, M., & Peng, J. (2019). Serum Homocysteine, cystatin C as biomarkers for progression of diabetic nephropathy. *Pteridines*, *30*(1), 183–188.

Yang, J., Fang, P., Yu, D., Zhang, L., Zhang, D., Jiang, X., Yang, W. Y., Bottiglieri, T., Kunapuli, S. P., Yu, J., Choi, E. T., Ji, Y., Yang, X., & Wang, H. (2016). Chronic Kidney Disease Induces Inflammatory CD40 + Monocyte Differentiation via Homocysteine Elevation and DNA Hypomethylation. *Circulation Research*, *119*(11), 1226–1241. https://doi.org/10.1161/CIRCRESAHA.116.308750

Yaribeygi, H., Sathyapalan, T., Atkin, S. L., & Sahebkar, A. (2020). Molecular mechanisms linking oxidative stress and diabetes mellitus. *Oxidative Medicine and Cellular Longevity*, *2020*.

Yi, F. (2007). dos Santos EA, Xia M, Chen QZ, Li PL, Li N. *Podocyte Injury and Glomerulosclerosis in Hyperhomocysteinemic Rats. Am J Nephrol*, *27*, 262–268.

Zhang, J., Zhang, R., Wang, Y., Li, H., Han, Q., Wu, Y., Wang, T., & Liu, F. (2019). The Level of Serum Albumin Is Associated with Renal Prognosis in Patients with Diabetic Nephropathy. *Journal of Diabetes Research*, *2019*, 1–9. https://doi.org/10.1155/2019/7825804.