Association of Chymase and Angiotensin II with Early Detection of Diabetic Nephropathy in Patient with T2DM

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Abstract
Diabetes mellitus(DM) is a metabolic disease with the mean characteristic is hyperglycemia resulting from defects in insulin action, insulin secretion, or insulin resistance, it always jointed with long-term harm, dysfunction, and failure of organs, particularly the heart, nerves, eyes, kidneys, and blood Objective:- This study aims to investigate the association between chymase and angiotensin II levels in type 2 diabetic patients with early and chronic diabetic nephropathy.Subjects and Method:- A case-control study of individuals with type 2 diabetes was conducted from November 2021 to May 2022. This study was conducted on 62 patients with type 2 diabetes who attended Imam Hussein Medical City in Karbala. And 60 subject as a healthy control group. The patients were divided into three groups according to the ratio of their urinary albumin to creatinine ratio (ACR): Normoalbuminuria (n=22) the ACR was less than 30, Microalbuminuria(n=20) the ACR (30-299) and Macroalbuminuria (n=20), ACR was greater or equal to 300. All biochemical parameters measured by spectrophotometer, chymase and Angiotensin II measured by ELISA. Result: - The study of the Comparison between type 2 Diabetes Mellitus with Diabetic Nephropathy groups for Biochemical Parameters showed In (HDL, LDL, FBS, CHOL, HbA1c, Macroalbuminuria, TG, VLDL, blood urea, serum creatinine, and Angiotensin II), there were statistically significant differences between all groups, as well as in the Chymase it was statistically significant differences between the first and third and second and third groups.The results showed that there was a significant positive correlation between the blood levels of Microalbuminuria and ACR Opposite Chymase, and ANGII levels of the patients. Conclusion: - Increased levels of both chymase and ANG II levels play a role in the pathogenesis of diabetic nephropathy, the current study demonstrates that higher HbA1c% and poor glycemic control are closely associated with a greater likelihood of increased levels of chymase and ANG II and thus increased risks and complications of diabetic nephropathy in patients with type 2 diabetes mellitus.
Keyword: Diabetes mellitus, diabetic nephropathy, Chymase, Angiotensin II

1. Introduction

Diabetes mellitus(DM) is a metabolic disease with the mean characteristic is hyperglycemia resulting from defects in insulin action, insulin secretion, or insulin resistance, it always is jointed with long-term harm, dysfunction, and failure of organs, particularly the heart, nerves, eyes, kidneys, and blood [1] Diabetes mellitus is a chronic metabolic disease with a world prevalence of 8.4% [2]. The prevalence of DM in Iraq is very high and approaching 20% which is comparable to that in some other Middle East countries [3] In 2017, the mortality rate due to diabetes reached 10.7% in adult patients (20-79years) Around 1.4 million of Iraqis have diabetes Reported T2DM prevalence in Iraq ranges from 8.5% to 13.9% [4].

Uncontrolled hyperglycemia is associated with the development of DM microvascular (neuropathy, retinopathy, and nephropathy) and macrovascular (stroke, ischemic heart disease, and peripheral vascular disease) complications [5].

Type 2 Diabetes Mellitus: accounts for 90- 95 % of diabetes mellitus also
previously referred to as non-insulin-dependent diabetes or adult-onset diabetes.

Mostly present in adulthood, although it can be diagnosed in teens and
young adults due to the high prevalence of obesity[6]

Diabetic nephropathy accounts for 40 % of new cases of end-stage renal disease [7] Is an increment in protein excretion in urine with Microalbuminuria considered as an early stage of DNP in which a small increase in urinary albumin excretion (UAE) also called incipient DNP. As the disease advanced with the presence of Macroalbuminuria with overt DNP[8] Mast cells (MCs), fibroblasts, and vascular endothelial cells are the main sources of Chymase. Inflammatory signals, tissue damage, and cellular stress cause MC Chymase to be released into the extracellular. Chymase is a significant extravascular generator of angiotensin II (ANG II) [9] Inflammation and fibrosis are typical symptoms of CKD, regardless of the cause. Excessive innate and adaptive immune responses, as well as infiltration of inflammatory cells and the production of cytokines, characterize inflammation. Extracellular matrix (ECM) proteins eventually replace normal tissue architecture in fibrosis, preventing the normal functioning of

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specialized kidney cells such as tubular epithelial cells, podocyte, and mesangial cells in the glomeruli, and vascular endothelial Cells [10]

Renal fibrosis is characterized by tubulointerstitial fibrosis and glomerulosclerosis and is frequent in chronic kidney disease (CKD) that progresses to end-stage renal disease (ESRD). Renal fibro genesis involves the activation of inflammatory cells inside the glomerulus or renal interstitium, causing them to generate fibrogenic and inflammatory cytokines, promote cellular phenotypic change, and create extracellular matrix components [11]. Renal damage that lasts for a long time results in a fibrous scar and kidney failure. In renal fibrosis, TGF- is an established cytokine. [12] Extracellular matrix (ECM), which is largely made up of collagen, accumulates in the kidneys, causing fibrosis. MMPs are proteins that break down collagen and ECM proteins and are generated in a variety of organs, including the kidney. MMPs are thought to have both anti- and pro-fibrotic properties. The majority of MMPs are secreted as pro-MMPs that must be cleaved to be activated [13].

2. Subject and material

A case-control study was conducted for individuals with type 2 diabetes from November 2021 to May 2022. This study was conducted on 62 patients with type 2 diabetes who attended Imam Hussein Medical City in Karbala and 60 as a healthy control group, and the patients were divided into three groups according to their urinary albumin ratio to creatinine ratio (ACR), they were matched in their sex and age with control group. Control Group they were collected from the same center in Imam Hussein Medical City hospital, 12 of them were female and 16 were males. Their age ranged from (39-70) years.

Inclusion Criteria T2DM and Age (40-75). Exclusion Criteria Patients with T1DM Patients with a statin drug, or angiotensin-converting enzyme inhibitors(ACEI), Patient T2DM with antihyperglycemic drugs, Pregnant women, Patients with urinary tract infection, Patient with chemotherapy, and Systemic illness.

Venous blood samples were obtained from all studied subjects after overnight fasting by use of disposable syringes. Five to eight milliliters of blood were obtained from each subject and divided into two parts:

A. Venous blood (2mls) from each drawn sample was saved in a tube containing EDTA as an anticoagulant to be used in the measurement of the HbA1c level [14].

B. the remaining blood samples were allowed to clot at room temperature and then centrifuged at 3000 rpm for 10 minutes. Fasting blood sugar, blood urea, Lipid profile, and serum creatinine were measured by colormetric methods; remaining Sera were transferred carefully and stored at -17°C until analysis time in suitable serum tubes for Chymase and angiotensin II levels measurements by ELISA method test based on the quantitative sandwich principle (BT LAB-china), and the urine sample was collected from each subject, for determination of microalbumin in urine by spectrophotometer kit(Humane-Germany),

3. Results

The Statistical Package for the Social Sciences (SPSS) model 23 statistic program was used to examine the data of study. Data presented as mean ± SD (standard deviation). Independent T-test statistics were applied for parameters to compare between patients and controls groups and Anova test to compare between patients groups It was considered to be significant when p≤0.05.

The control group included 28 samples of healthy subjects, the number of females was 12 (42.9%), the number of males was 16 (57.1%), and their ages ranged between (39-70) years. Table1 shows the demographic characteristics of patients and control group.

As well as they were divided according to the Ratio of albumin to creatinine (ACR) into three groups: the first group (<30), whose number is 22 (35.5%), the second group (30-299), whose number is 20 (32.3%), and the third group (≥ 300), their number is 20 (32.3%), as shown in Table 1 and figure 2.

The ratio between males and females is equal in the group of patients and the control group, and there were no statistically significant differences between them in age and body mass index (BMI). The differences between diabetic patients and control groups were significant (P≤0.01) in the Duration of the disease and Albumin - Creatinine Ratio (ACR) as shown in Table 2.

This study showed a highly significant (P≤0.01) in fasting blood sugar (FBS), glycated hemoglobin (HbA1c), blood urea, Serum Creatinine, Microalbuminuria, albumin-creatinine ratio (ACR), cholesterol (CHOL), triglyceride (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), very-low-density lipoprotein(VLDL), Chymase and AngiotensinII. Between diabetic patients and the control groups.

To assess the relationship between the levels of Microalbuminuria, albumin-creatinine ratio (ACR), Chymase, and ANGII levels in patients, the linear regression analysis was used to evaluate the data Table 5{Radi, 2022 #21}.

The study revealed a positive linear regression between Chymase and ANGII with Microalbuminuria (P<0.001, r=0.57), (P<0.001, r=0.82) respectively. And a positive linear regression between Chymase and ANGII with ACR, (P<0.001, r=0.70), (P<0.001, r=0.91) respectively.

4. Discussion

Diabetes mellitus has long been recognized as a major health problem with far-reaching consequences, not only for its adverse health impact on individuals but also for its economic burden on the health care system and society at large. It is the

sixth leading cause of death by disease in the United States, accounting for almost 18% of all deaths in people over 25 years of age, and it is the leading cause of the end-stage renal disease (ESRD) [15] The result of the demographic study showed that there were more patients at age ≥ (45-60) years old (41.9 %) compared to the control group (28.6%) Table (1and 2), this study is in agreement with the results of a study conducted in Saudi Arabia in 2018 [16]. The incidence of males is 56.5% higher than that of females 43.5%, and the result of this study was in agreement with the results of a study conducted in Palestine [17],the reason for this may attributed to the daily effort that men are exposed in compare to women. As shown in tables (1and 2). Because of insulin resistance, which is commonly associated with this clustering of metabolic factors, frequently precedes the onset of type 2 diabetes [18]. The percent of patients who have hypertension was 62.9% and this percent related to the WHO criteria that were found the presence of hypertension in patients with diabetes mellitus was 40% [19]. The elevation in blood pressure in patients compared with the control group, hypertension in type 2 diabetes occurs from insulin resistance and the cause of hypertension in insulin resistance contribute to volume expansion because insulin enhances proximal tubular sodium reabsorption and endothelial dysfunction [20]{Teixeira, 2021 #32}

The higher Microalbuminuria in patients compared to the control group as shown in Table 3 because Microalbuminuria is a sign or predictor of diabetic nephropathy and cardiovascular disease in patients with type 1 and type 2 diabetes mellitus [21], Hyperglycemia is a critical factor in the development of diabetic nephropathy due to its effects on glomerular and mesangial cells, but alone it is not a causative agent. Melanocytes are essential for maintaining the glomerular capillary structure and for modulating glomerular filtration via smooth muscle activity. Hyperglycemia is associated with increased proliferation and hyperplasia of mesangial cells, as well as increased matrix production and basement membrane thickness, and consequently increased Microalbuminuria [22]. In this study, the prevalence of Microalbuminuria was 65.5% (Table 4) and this is related to other percentages obtained from other studies in Saudi Arabia where it was 45.6% [23], and in the United Arab Emirates, it was 61.2% [23]. Other studies revealed that the prevalence of Microalbuminuria is less than this percentage in this study A study in Tanzania revealed that the incidence of Microalbuminuria in type 2 diabetes is 9.8% [24], this variation in prevalence can be attributed to factors such as differences in population, method of urine collection or difference in racial susceptibility, differences in race, poor health and education settings.

The significant elevation in Chymase levels between healthy people and patients, where the results were (1.64±0.39) and (3.25±1.55) ng/ml, respectively ($p<0.001$), This is in agreement with what was obtained by other studies[25, 26] which found the reason is that diabetes significantly increases the effect of inflammatory cytokines associated with mast cells, and thus increases Chymase, Chymase is an alternative pathway for the angiotensin-converting enzyme in angiotensin II (Ang II) formation, and its expression stimulated increased in human with diabetic kidney disease, and human mesangial cells (MCs), with high glucose in blood [25], These authors demonstrated that chymase promotes cell proliferation and collagen synthesis and increases fibroblasts by mechanisms dependent on TGF-b1 and independent of Ang II[27]. When comparing Chymase with the ACR groups in Table 4, the current study found that the Macroalbuminuria group had the highest Chymase level, which was 4.80 ± 1.96, while the Microalbuminuria group reached 2.76± 0.19, and the Normoalbuminuria group reached 2.28 ± 0.18, where the study showed that the Chymase rate increases with ACR increase and this is in agreement with other study[28], which found that due to the association of mast cell infiltration into the kidneys with proteinuria and interstitial fibrosis in various kidney diseases [28].

The current study found As shown in table (3) that there were significantly increase in Angiotensin II levels in patient when compared to healthy (41.25 ± 17.03), (16.98± 3.55) ng/l, respectively, ($p<0.001$), This is in agreement with what was obtained by other studies[29, 30] that showed Local adipose renin angiotensin system (RAS) participates in obesity-associated metabolic alterations. Emerging evidence indicates that the development of insulin resistance and type2 diabetes was associated with the augmented expression of ANG II generating enzymes and RAS activation in adipose tissue[31], In addition, this result suggests that high glucose (HG) level in blood lead to increase in intracellular ANG II generation involves the ACE pathway. Moreover, mast cell (MC) exposed to HG has increased intracellular renin activity paralleled by an increase in the angiotensinogen gene transcription[30]. When comparing Angiotensin II with the ACR groups in Table 4, the current study found that the Macroalbuminuria group had the highest Angiotensin II level, which was 62.33± 12.18, while the Microalbuminuria group reached 36.72± 5.05, and the Normoalbuminuria group reached 26.21 ± 2.54, where the study showed that the Angiotensin II rate increases with ACR increase and this is in agreement with a study[32] that found in experiment, glucose stimuli and ANG II rapidly increased the vascular endothelial growth factor (VEGF) synthesis in proximal tubular epithelial cells. This finding may provide a clue to understanding why the proximal tubule participates actively in the regulation of intrarenal VEGF synthesis[32] The clinical impact of this increased expression of VEGF, is caused by high glucose concentrations and ANG II in proximal tubule cells, is not known. Although it has been suggested that VEGF acts as a potential mediator of glomerular hyperfiltration and albuminuria in the

glomerulus, there is also strong evidence for VEGF acting as an important endothelial cell angiogenic, survival, and trophic factor[33].

5. Conclusions

The poor glycemic control associated with increase of micro and macro albuminuria leading to increase stimulation of chymase that lead to elevation of angiotensin II and increase the risk of hypertension in patient with T2DM and increase of diabetic nephropathy.

6. Acknowledgment

All thanks to the studied subjects for their acceptance in participation in this stud.

Table 1: Demographic characteristics of diabetic nephropathy groups and Control groups

Variables	Control group	Diabetic group
Total Number	28(100%)	62(100%)
Age (years)		
(35-45) years	9 (32.1%)	11 (17.7%)
(46-60) years	8 (28.6%)	26 (41.9%)
(>60) years	11 (39.3%)	25 (40.3%)
Sex		
Male	16(57.1%)	35(56.5%)
Female	12(42.9%)	27(43.5%)
Body mass index (BMI) Kg/m²		
Underweight <18	1 (3.6%)	4 (6.5%)
Normal weight 18-24.9	8 (28.6%)	10 (16.1%)
Over weight 25- 29.9	14 (50.0%)	21 (33.9%)
Obesity ≥30	5 (17.9%)	27 (43.5%)
Albumin-creatinine ratio(µg/mg)		
< 30	28 (100%)	22 (35.5%)
30-299		20(32.3%)
≥ 300		20(32.3%)
Hypertension		
Present	0	39 (62.9%)
Absent	0	23 (73.1%)

Table 2: The association of study groups by different variables

Variables	Control group	Diabetic group	χ2	p-value
Total Number	28(100%)	62(100%)		
Age (years)				
(35-45) years	9 (32.1%)	11 (17.7%)	2.71	0.257
(46-60) years	8 (28.6%)	26 (41.9%)		
(>60) years	11 (39.3%)	25 (40.3%)		
Sex				
Male	16(57.1%)	35(56.5%)	0.04	0.951
Female	12(42.9%)	27(43.5%)		
Body mass index (BMI) Kg/m²				
Underweight (<18)	1 (3.6%)	4 (6.5%)	6.65	0.084
Normal weight (18-24.9)	8 (28.6%)	10 (16.1%)		
Over weight (25- 29.9)	14 (50.0%)	21 (33.9%)		
Obesity (≥30)	5 (17.9%)	27 (43.5%)		
Albumin-creatinine ratio(µg/mg)				
Control	28 (100%)	0	90.00	0.001
< 30	0	22 (35.5%)		
30-299	0	20(32.3%)		
≥ 300	0	20(32.3%)		
Duration of the disease(year)				
Control	28(100%)	0	90.00	0.001
<5 year	0	13 (21.0%)		
5-10	0	16 (25.8%)		
>10	0	33(53.2%)		

Table 3: Comparison of biochemical characteristics between patients groups and Control group included in the study

Group Parameters	CONTROL (Mean ± S.D) n= 28	RANG (Min-Max)	Diabetic patients (Mean ± S.D) n= 62	RANG (Min-Max)	p-value
age(year)	55.82 ± 11.70	39.00-70.00	57.96 ± 10.25	38.00-75.00	0. 382
BMI(Kg/m2)	26.58 ± 3.61	16.75-32.50	28.00 ± 4.41	17.17-36.26	0.141
Duration of the Disease(year)	0	0.00	11.27±6.17	2.00-23.00	0. 001
FBS(mg/dl)	94.13± 8.02	82.00-110.00	175.14± 23.75	124.00-211.00	0. 001
Blood urea (mg/dl)	26.84 ± 6.79	18.50-40.00	73.77± 41.79	30.30-175.00	0. 001
S. creatinine (mg/dl)	0.70 ± 0.14	0.45-1.10	2.14± 1.29	0.90-5.50	0. 001
HbA_{1c} (%)	5.45 ± 0.36	4.50-5.80	8.92± 1.83	6.20-12.90	0. 001
Microalbuminuria (mg/l)	13.51± 2.18	10.50-19.00	100.93± 66.11	5.50-205.00	0. 001
Albumin-creatinine ratio (µg/mg)	15.77±2.26	13.00-21.50	142.84 ± 94.41	11.00-320.00	0. 001
CHOL(mg/dl)	160.03 ± 11.28	137.00-185.00	200.23 ± 27.25	148.20-245.00	0. 001
TG(mg/dl)	109.68 ± 11.82	86.50-133.00	176.99± 51.83	76.40-265.30	0.001
HDL(mg/dl)	38.84± 5.11	34.00-53.00	35.13 ± 9.31	24.30-59.50	0. 001
LDL(mg/dl)	99.23± 10.55	79.30-116.90	130.35± 23.75	75.10-172.60	0. 001
VLDL(mg/dl)	21.91± 2.35	17.30-26.60	35.50± 10.21	15.28-53.00	0. 001
Chymase(ng/ml)	1.64 ± 0.39	0.66-2.09	3.25± 1.55	2.04-8.27	0. 001
AngiotensinII(ng/l)	16.98 3.55	8.28-21.68	41.25 ± 17.03	21.88-80.01	0. 001

. t-test (normal distribution) and Mann-Whitney Test (abnormal distribution), P≤0.01 highly significant difference, P≤0.05 significant difference, S.D: Standard Deviation.

Table 4: The Comparison between types 2 Diabetes Mellitus with Diabetic Nephropathy groups for Biochemical Parameters			
ACR Group Parameters	Mean ± S.D		
	<30 n= 22	30-299 n= 20	≥ 300 n= 20
Age (year)	54.09 ± 11.70 a	56.75 ± 9.47 ab	63.45± 6.79 b
BMI(Kg/m^2)	25.18 ± 4.88 a	29.36 ± 3.96 b	29.74± 2.47 b
Duration of Disease	5.63 ± 3.69 a	11.40± 4.50 b	17.35 ± 3.39 c
FBS(mg/dl)	148.88 ± 14.48 a	178.30± 5.16 b	200.78 ± 6.89 c
Blood urea (mg/dl)	36.80 ± 3.43 a	62.70± 13.44 b	125.52 ± 29. 72 c
S. creatinine (mg/dl)	1.11 ± 0.09 a	1.55± 0.29 b	3.35 ± 0.97 c
HbA_1c (%)	7.07 ± 0.62 a	8.68 ± 0.35 b	11.19± 0.93 c
Microalbuminuria(mg/l)	14.94 ± 4.23 a	99.54±36.03 b	196.91 ± 41.78 c
CHOL(mg/dl)	176.64± 19.80 a	195.19± 11.24 b	231.24 ± 11.89 c
TG(mg/dl)	117.43 ± 28.19 a	187.71± 8.81 b	231.77 ± 16.84 c
HDL(mg/dl)	43.44 ± 10.14 a	30.59± 4.17 b	30.54 ± 4.85 b
LDL(mg/dl)	111.39 ± 19.38 a	127.21± 13.40 b	154.33 ± 12.51 c
VLDL(mg/dl)	23.18±5.55 a	37.51±1.79 b	46.53±3.36 c
Chymase(ng/ml)	2.28 ± 0.18 a	2.76± 0.19 a	4.80 ± 1.96 b
AngiotensinII(ng/l)	26.21 ± 2.54 a	36.72± 5.05 b	62.33± 12.18 c

1. One-Way ANOVA Test (normal distribution) and Kruskal-Wallis Test (abnormal distribution), S.D: Standard Deviation.
2. Different letters in the same raw refer to significant differences ($p \leq 0.05$)

Table (5): linear regression analysis among Microalbuminuria, albumin-creatinine ratio (ACR), Chymase, and ANGII levels in patients								
parameter	Microalbuminuria		ACR		Chymase		ANGII	
	r	p	r	P	r	p	r	p
Microalbuminuria			0.93	<0.001	0.57	<0.001	0.82	<0.001
ACR	0.93	<0.001			0.70	<0.001	0.91	<0.001
Chymase	0.57	<0.001	0.70	<0.001			0.90	<0.001
ANGII	0.82	<0.001	0.91	<0.001	0.90	<0.001		
parameter	Microalbuminuria		ACR		Chymase		ANGII	
	r	p	r	P	r	p	r	p
Microalbuminuria			0.93	<0.001	0.57	<0.001	0.82	<0.001
ACR	0.93	<0.001			0.70	<0.001	0.91	<0.001
Chymase	0.57	<0.001	0.70	<0.001			0.90	<0.001
ANGII	0.82	<0.001	0.91	<0.001	0.90	<0.001		

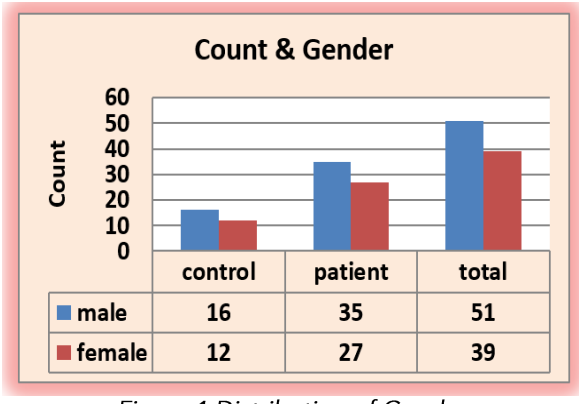

Figure 1 Distribution of Gender

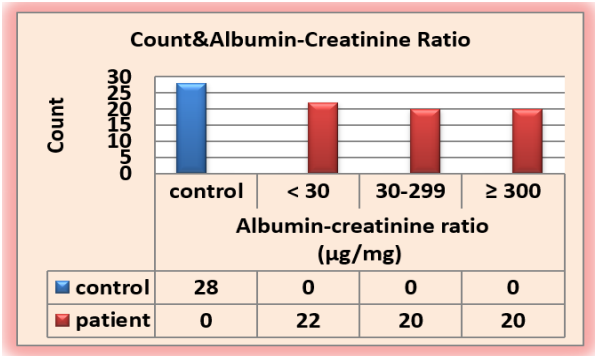

Figure 2 Distribution of Albumin/Creatinine Ratio

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