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ORIGINAL ARTICLE

Comparison the Role of Some Novel Biomarkers in Predicting Diabetic Nephropathy Between Diabetic Type 2 and Chronic Kidney Diseases Patients in Kirkuk City

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ABSTRACT

Background: Diabetes mellitus most dangerous metabolic disorder and have high morbidity and mortality complications, the most DM complication is diabetic nephropathy. Diabetic nephropathy(DN) is an irreversible progressive disorder characterized by increased urine albumin excretion and/or impaired GFR. DM causes about 50 % of kidndiseasesase. An increase in prevalence of diabetes is projected to lead to a significant increase in patients with end-stage renal disease (ESRD) requiring renal replacement therapy (RRT), and up to 50% of the dialysis population is diabetic. We study 30 Diabetic patients with Chronic kidney diseases with hemodialysis ,30 diabetic patient with chronic kidney disease with out hemodialysis , 15 patient with diabetes only , and 15 control group .we measured kidney injury molecule-1 , liver type fatty acid binding protein ,zinc alpha 2 glycoprotein , serum creatinine ,uric acid .blood urea and random blood sugar .results showed that highest KIM-1 concentration in diabetic nephropathy with hemodialysis group (2.325± 1.095) followed by control group (1.842 ±0.866 ab)and followed with diabetes group (1.572 ±0.752 b) and lowest mean level was diabetic nephropathy without hemodialysis group (1.344 ±0.623 b) with highly significant differences ($p < 0.001$) of KIM-1 among groups .The highest mean level of L-FABP was recorded in diabetic nephropathy with hemodialysis group (254.6 ± 29.6 a) followed by the control group (235.8 ± 28.9) and followed by diabetic group (9210.3 ± 28.7 ab)and the lowest mean level was diabetic nephropathy without hemodialysis group (149.5 ±25.9 a) with significant difference ($P<0.002$) of L-FABP among groups .The study showed that the highest mean level of ZAG recorded in diabetic nephropathy with hemodialysis group (140.1 ± 23.6 a) followed by control group(130.9 ±11.5 a)and the lowest mean level was in diabetes group (88.3 ± 14.0 b) and diabetic nephropathy without hemodialysis group (87.4 ± 19.60 b) respectively with highly significant difference ($P<0.0003$).

Aims: Role of biomarkers (KIM-1, L-FABP, ZAG) in detection early development of diabetic nephropathy .Correlation between biomarkers (KIM-1, L-FABP, ZAG) and progression of diabetic nephropathy to end stage of renal disease .Estimate how much relationship between biomolecules (creatinine , urea and uric acid) with diabetes and its complication

Methodology :The study included 90 individuals from Kirkuk city / Blood sample collected from patient and analysis was conducted to estimate concentration using Enzyme Linked Immunosorbent Assay (ELISA),and biochemicals using spectrophotometers .

Keywords: Diabetes, diabetic nephropathy , chronic kidney disease ,kidney biomarkers

INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels (1). Diabetic nephropathy (DN) is irreversible progressive disorder characterized by increased urine albumin excretion and/or impaired GFR. There are different pathophysiological mechanisms contribute to the development and progression of DN such as hyperfiltration, synthesis of growth factors, cytokines, and release of reactive oxygen species (ROS) (2). DM it causes about 50 % of kidney disease. increase in prevalence of diabetes is projected to lead to a significant increase in patients with end-stage renal disease (ESRD) requiring renal replacement therapy (RRT), and up to 50% of the dialysis population is diabetic (3). There are many biomarker that help to early detect and determine the progression of the DN, to prevent development or reduce it is complication.

kidney injury molecule-1 (KIM-1) It is a type I transmembrane glycoprotein with two extracellular domains expressed at the apical membrane of the proximal tubular cells but also expressed in the glomerular epithelial cells. Studies confirmed KIM-1 as a biomarker of acute kidney injury or chronic kidney diseases (CKD) its concentration is markedly increased following kidney injury , the extracellular domains of KIM-1 separate from the cell surface and enter the urine . KIM-1 expression is low in normal kidneys but is significantly increased in proximal tubule cells following AKI. (4) (5). In type 2 diabetes, urinary KIM-1 levels increased from the normoalbuminuria to the last macroalbuminuria, predicting the progression of DN . Urinary Kim-1 also was high in normoalbuminuric diabetics before reduction in GFR, indicating early diabetic kidney injury (6).

Liver type fatty acid binding protein(l-FABP) is expressed in proximal tubular cells in the kidney and secreted into the urine when tubular damage occurs. (7) . l-FABP binds fatty acids and transports them to mitochondria or peroxisomes, and it contributes to intracellular fatty acid homeostasis (8). It has been reported that expression of L-FABP in the proximal tubules is increased by ischemia or oxidative stress that causes tubulointerstitial damage ,furthermore, urinary L-FABP has been used to predict deterioration of renal function in diabetic patients (9). Study results demonstrated that u-LFABP levels are increased gradually with increasing severity of DN in subjects with type 2 diabetes indicating tubular damage. Macroalbuminuric subjects had higher levels of u-LFABP as compared to normoalbuminuric and microalbuminuric subjects. (10).

Zinc-α2-glycoprotein (ZAG, also called AZGP1) is a novel identified 43 kDa adipokine that has been demonstrated to play an important role in the regulation of body weight, glucose, and lipid metabolism (11) . Evidence supports that the ZAG levels are lower in obese and insulin resistant (IR) individuals , and that there is an inverse relationship of ZAG with body mass index (BMI) and IR (12). Association of serum ZAG with multiple factors including renal function, cachexia (relating to body weight and BMI) and/or inflammation (relating to CRP) led to the result. (13) . ZAG normally filtered in the glomerulus and thereafter cleared by the proximal tubule through reabsorption and lysosomal degradation (14) . Therefore, circulating ZAG significantly accumulates in patients with disrupted renal function and acute or chronic renal failure. (15)

MATERIAL AND METHODS

The study included 90 individuals from Kirkuk city / Iraq ;15 Diabetic type II patients, 30 Diabetic type II patients with Chronic kidney diseases without hemodialysis , 30 Diabetic type II patients with Chronic kidney diseases with hemodialysis,15Control (apparently Healthy subjects). Blood sample collected from patient and analysis was conducted to estimate concentration of KIM-1,ZAG,L-FABP using Enzyme Linked Immunosorbent Assay (ELISA),and biochemicals tests such as serum creatinine ,blood urea ,RBS and uric acid using spectrophotometers .Results were statically analysed using SPSS with data analysed using a one way contrast analysis of ANOVA followed by a multi-range Duncan test at a probability ($p<0.05$) to compare search groups **.**

RESULT

The results showed increase in concentration of biomarkers (ZAG, KIM-1and L-FABP) in ESRD group comparing to other study groups.at the probability ($P<0.05$) as shown in table (1) and figures (1,2,3,4,5,6,7,8).

Table 4-1: means (SD) of biomarkers for study groups

Groups parameter	diabetic nephropathy with hemodialysis	diabetic nephropathy without hemodialysis	Diabetes	Control
KIM-1	2.325±1.095 a	1.344±0.623 b	1.572 ± 0.752 b	1.842±0.886 ab
L-FABP	254.6±29.6 a	149.5±25.9 b	210.3±28.7 ab	235.8±28.9 a
ZAG	140.1± 23.6 a	87.4±19.60 b	88.3 ±14.0 b	130.9 ±11.5 a
Uric Acid	6.520 ±1.372 a	6.317 ±1.400 a	5.353± 1.800 b	5.800 ±1.154 ab
creatinine	8.3270±1.401 a	2.3050±0.383 b	0.6867±0.1237 c	0.6521±0.1182 c
Urea	123.80 ±14.47 a	93.97 ±12.61 b	26 .27 ±8.44 c	21.86 ±5.61 c
RBS	260.1 ±12.34 a	280.0 ±116.1 a	289.5 ±11.1 a	96.9 ±12.70 b
BMI	26.1 ± 4.4 a	28.7±4.7 ab	29.5± 4.4 b	23.1± 4.7 c

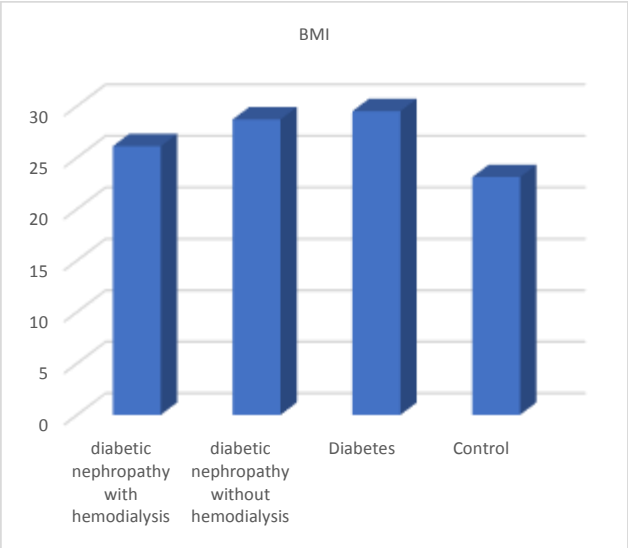

Figure 1: Mean(±SD) of BMI among study groups

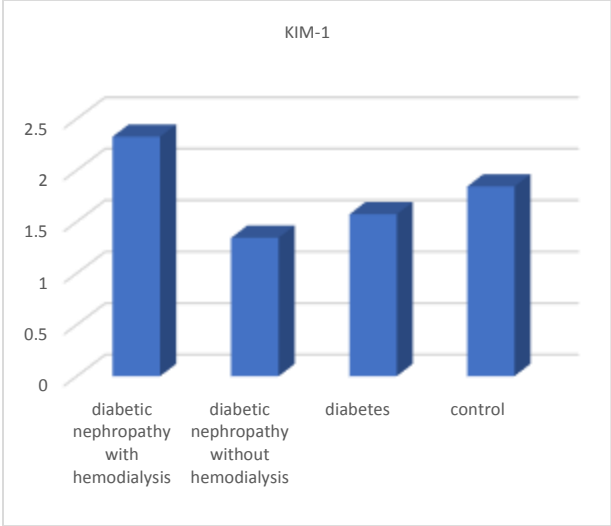

Figure 2: show mean (±SD) of KIM-1 among study groups

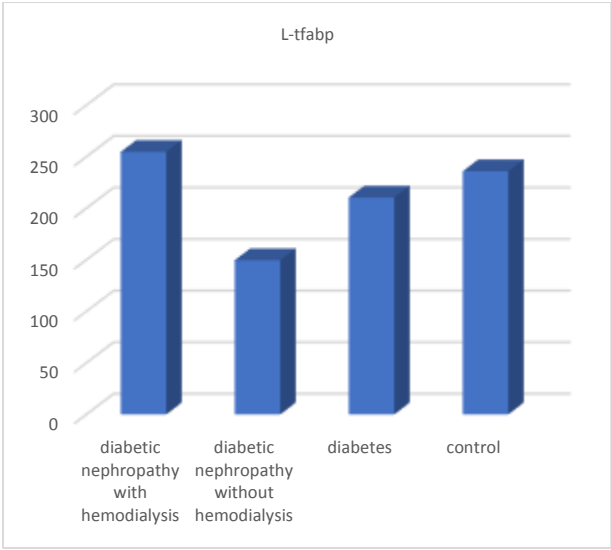

Figure 3: show the mean (±SD) of L-FABP concentration among study groups

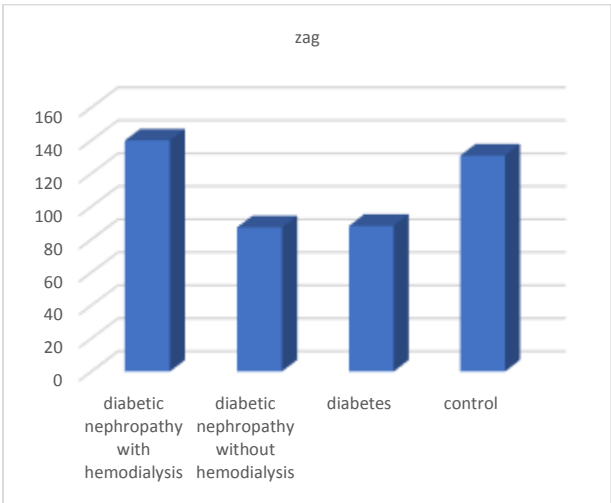

figure 4: show the mean (±SD) of ZAG concentration among study groups

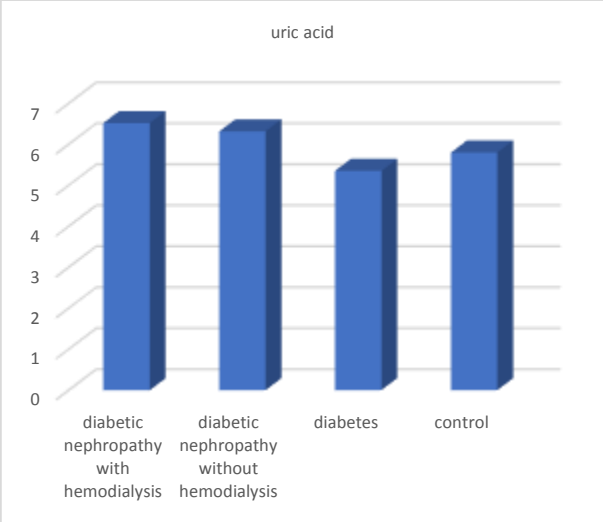

Figure 5: show mean (±SD) of uric acid concentration among study groups

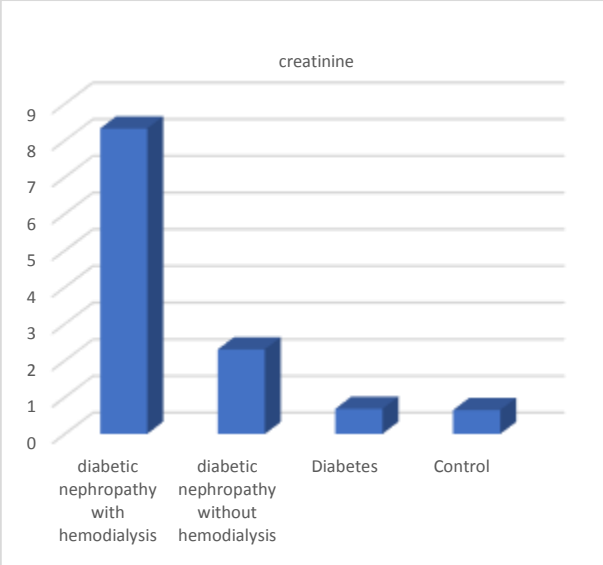

Figure 6: mean (±SD) of creatinine in study groups.

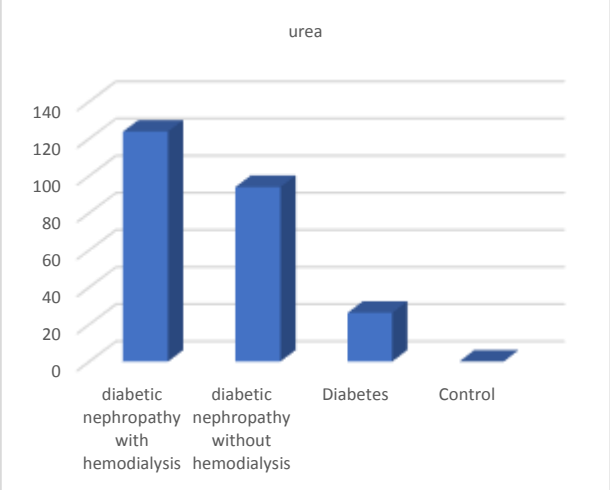

Figure 7: show mean (±SD) of urea level among study groups

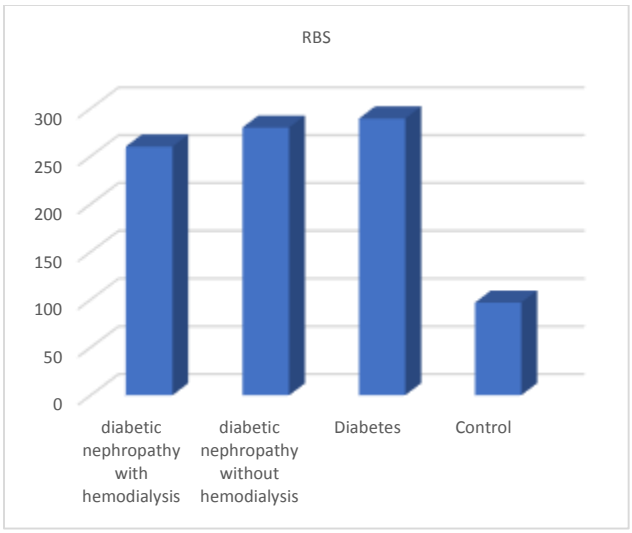

Figure 8: mean (±SD) of sugar among study groups .

DISCUSSION

This study include 30 patients with diabetic nephropathy and receiving hemodialysis ,30 patients with diabetic nephropathy without haemodialysis ,15 patients with diabetes only and 15 control group .

The evidence suggests that the tubulointerstitial damage as well as glomerular damage contributes to a decline in renal function. We used three biomarkers in our study to estimate how much these biomarkers are useful in predicting and progression of diabetic nephropathy. We use u-LFABP and KIM-1 has been demonstrated to be a marker of tubular damage and ZAG is considered to be a marker for glomerular damage .we use other parameters such as creatinine ,urea ,random blood sugar and uric acid to help us to determine the state of the study groups .we found a positive correlation between these biomarkers (ZAG,KIM-1,L-FABP) and ESRD patients we record high concentrations in diabetic nephropathy with hemodialysis groups compared to control groups

Kidney injury molecule 1 (KIM1) is a marker of tubular damage in various chronic kidney diseases. This type 1 cell membrane glycoprotein is expressed on the apical membrane of proximal tubule cells and is involved in the phagocytosis of damaged cells in the proximal tubules (16). KIM-1 has evolved as a marker of proximal tubular injury, the hallmark of virtually all proteinuric, toxic, and ischemic renal diseases. (17)

In our study diabetic nephropathy with hemodialysis group showed the highest concentration of KIM-1 compared to the control group this is because highly damaged renal proximal tubule may lead to detach KIM-1 from epithelial and excrete it to urine. Urinary KIM-1 levels were also significantly higher among patients who progressed from macroalbuminuria to late-stage CKD, but it did not predict progression to end-stage renal disease independently of albuminuria. (18)

While in diabetic nephropathy without hemodialysis group there is no elevation in the concentration of KIM-1 although many studies suggest that KIM-1 increases during kidney disease, this may be because patients receiving treatment that affected the KIM-1 concertation, patients undergoing classical diabetic treatment recorded a significant decrease in KIM-1 as compared with the diabetic persons. Inflamed kidney patients that undergo treatment recorded a significant decrease in KIM-1 (19) antiproteinuric drugs also decrease uKIM-1level (20).

Low level may be because the renal tubule may be not damaged and there is no KIM-1 excrete to urine from tubular epithelial. Renal KIM-1 correlated positively with renal damage, and negatively with renal function (21) . there is no increase in

KIM-1 concentration in diabetes groups this indicates there is no damage to the renal tubule yet.

L-FABP is expressed in proximal tubular cells in the kidney and secreted into the urine when tubular damage occurs. (7). L-FABP binds fatty acids and transports them to mitochondria or peroxisomes, and it contributes to intracellular fatty acid homeostasis (8) . Indeed, high urinary L-FABP levels are associated with progression to end-stage renal disease (ESRD) or induction of hemodialysis in T2DM (22).this support our study result that there is positive correlation between L-FABP and ESRD so diabetic nephropathy with hemodialysis group show highest concentration of L-FABP compared to control group this indicating kidney dysfunction and renal tubulointerstitial damage.

u-LFABP level was significantly correlated with proteinuria and systolic blood pressure, but did not correlate with microalbuminuria. We could not conclude whether glomerular damage occurred first or tubular damage at an early stage of development (23). Therefore in diabetic nephropathy without hemodialysis group no increase in the level of L-FABP because kidney disease not because renal tubule but due to glomerular damage which not affect to L-FABP concentration.

urinary L-FABP, did not predict a decline in GFR in patients with DN with overt nephropathy and this support our result in diabetic nephropathy without hemodialysis group (24) . There is no increase in L-FABP concentration in diabetes group this is because there is no kidney damage happened yet.

CKD, and especially end-stage renal disease, are associated with increased plasma concentration of most adipokines. These increases are traditionally thought to result from passive accumulation due to a decreased renal clearance. (25)

ZAG because normally filtered in the glomerulus and thereafter cleared by the proximal tubule through reabsorption and lysosomal degradation. Circulating ZAG significantly accumulates when renal clearance is disrupted in patients with acute and chronic renal failure. (26) , but also elevate from overproduction by adipocytes stimulated by the uremic environment. (27).

Therefore in our study we found diabetic nephropathy with hemodialysis group patients have the highest level of zag because it is not cleared by the kidney normally because of renal failure . other studies showed that serum ZAG levels were elevated in chronic hemodialysis (CH) patients, suggesting a decrease in renal clearance (28).

ZAG acts as an initiator of the catalytic conversion of adenosine triphosphate to cyclic adenosine monophosphate (cAMP), which is the initial step of lipolysis by binding to beta-3-adrenoreceptors on adipocyte surfaces. (29). Studies have shown that the expression and circulating levels of ZAG are inversely correlated to adiposity. ZAG mRNA and protein expression are decreased or lost in adipose tissues of obese patients. ZAG stimulate lipolysis in adipocytes via activation of b3- adrenergic receptors (b3-ARs) and activation of the cAMP pathway(30). Most of the evidence supports that the ZAG levels are lower in obese and insulin-resistant (IR) humans and that there is an inverse relationship of ZAG with body mass index (BMI) and IR (31). In human visceral and subcutaneous fat ZAG expression is negatively associated with increased adiposity and the parameters of insulin resistance (32) . Circulating ZAG levels in patients with type 2 diabetes mellitus are negatively associated with IR. Similarly, a negative association between the ZAG mRNA level in adipose tissue and IR has also been verified. (33). There for many studies show that circulating ZAG levels are lower in patients with T2DM and also significantly lower in overweight/obese patients and were negatively correlated with BMI, waist circumstance, hip circumference, and fat mass. (34) (35)

In our study in diabetes group and diabetic nephropathy without hemodialysis group showed, low level of ZAG, because these two groups have high BMI and insulin resistance and inversely relationship of ZAG with BMI and diabetes (insulin resistance) explains why ZAG decreased in these two group . .low level also may be because renal clearance not fully disrupted and ZAG continuously cleared by the kidney.

CONCLUSION

In this study, we found that biomarkers have a correlation with ESRD and can predict progression to late stage kidney disease. ZAG recorded the highest level in diabetic nephropathy with hemodialysis patients because of renal clearance disruption, also KIM-1 and L-FABP record the highest level in diabetic nephropathy with hemodialysis because of tubular damage .

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