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RESEARCH ARTICLE

KIM-1 AND NGAL AS BIOMARKERS OF NEPHROPATHY IN TYPE II DIABETES.

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Abstract

Background: clinically detectable diabetic nephropathy is based on albumin excretion and glomerular filtration rate that mainly reflects the glomerular damage. New biomarkers have arisen with the promise of detecting kidney damage prior to the currently used markers. **Objectives:** to assess the usefulness of the urinary neutrophil gelatinase-associated lipocalin and kidney injury molecule-1 for the early diagnosis of diabetic kidney disease in type2 diabetes mellitus and to estimate their relation to the degree of nephropathy in comparison to the level of albuminuria and other parameters.

Design: cross sectional study.

Setting: the internal medicine department - al-zahraa university hospital and endocrinology department - helwan university hospital from 2014 / 2015.

Patients and methods: seventy diabetic patients were chosen and divided into 3 subgroups (normoalbuminuric, microalbuminuric and macroalbuminuric), compared to 19 healthy controls. For all participants, clinical examination, urine analysis, fasting and 2hours post prandial serum glucose, glycated hemoglobin a1c%, serum cholesterol, triglycerides, serum creatinine, urea, estimated glomerular filtration rate, urinary albumin to creatinine ratio, urinary kim-1 and ngal were done.

Results: both urinary ngal and kim-1 correlated positively with urinary albumin, disease duration and hba1c. Ukim-1 correlated negatively with estimated glomerular filtration rate. The cutoff point to differentiate the normoalbuminuric group from control for ukim-1 was 88.5ng/l. The diagnostic sensitivity was 91.6%, diagnostic specificity was 84%. At the cutoff value 3.25 µg/l for ungal the diagnostic sensitivity was 100%, diagnostic specificity was 89.4%.

Conclusion: ukim-1 and ungal are sensitive and specific markers for detection of diabetic nephropathy in patients with type2 diabetes mellitus.

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Introduction:-

Diabetic kidney disease is a syndrome characterized by gradual increasing albuminuria; worsening hypertension associated with decline in glomerular filtration resulting in end-stage renal disease.⁽¹⁾ Although diabetic nephropathy (DN) is thought to be a primary glomerular disease, the deterioration of function correlates with the degree of tubulointerstitial fibrosis which is suggested to be a better predictor of disease progression than the

severity of glomerular damage.⁽²⁾ Tubular injury with prolonged exposure to a variety of hemodynamic and metabolic factors may contribute in a primary way, rather than a secondary manner, to the development of DN.⁽³⁾ A growing of evidence indicates that some tubular biomarkers have good predictability for early diagnosis of diabetic nephropathy, such as kidney injury molecule-1(KIM-1) and neutrophil gelatinase-associated lipocalin (NGAL).⁽⁴⁾ Neutrophil gelatinase-associated lipocalin and urinary kidney injury molecule-1 are tubular damage markers that are reported as early detectors of acute kidney injury and they are investigated as early markers in chronic kidney disease patients.⁽⁵⁾ Kidney injury molecule-1 is a transmembrane glycoprotein that not expressed in normal proximal tubule cells but is up regulated in the proximal tubule during various forms of acute kidney injury, and also expressed in chronic kidney diseases, which is mainly characterized by tubulointerstitial changes.⁽⁶⁾ Urinary levels of KIM-1 were significantly higher in diabetic patients in comparison with non-diabetic subjects indicating the possible clinical utility of urinary KIM-1 as early complementary biomarker of DN. Neutrophil gelatinase-associated lipocalin is one of acute-phase proteins that belongs to a lipocalin family which is a family of proteins that is highly induced in inflammatory conditions, ischemia, and nephrotoxic injury of the kidneys.⁽⁷⁾ Serum NGAL is filtered by the glomerulus then degraded in the proximal renal tubule after capture by the megalin complex, and a minimal amount is excreted in urine. Its low molecular size, protease resistance and early appearance independent of GFR make it a convenient biomarker of kidney injury.⁽⁸⁾ Clinical and experimental studies of human biopsies with fibrotic damage or tubular necrosis revealed that, both uNGAL, uKIM-1 are good markers of active tubular damage and not tubular scarring. Therefore they are representative of the functioning tubular mass that can still be saved.⁽⁹⁾ Urine seems to be a good material for clinical diagnostics because it can be collected non-invasively, relatively stable and the procedure is fast and cost-efficient. The collection of blood is associated with the activation of proteases and generation of proteolytic breakdown products.⁽⁴⁾ This study aimed to assess urinary KIM-1 and NGAL as biomarkers for the early diagnosis and assessment of severity of diabetic nephropathy in Egyptian patients with type 2 diabetes mellitus (T2DM).

Patients And Methods:-

This case control cross-sectional study was conducted on 70 patients with T2DM fulfilling the American Diabetes Association criteria (2013)⁽¹⁰⁾ recruited from the Internal Medicine Department Al-zahraa University Hospital and Endocrinology Department of Helwan University Hospital from 2014/2015. Thirty of them were males and forty of them were females. Their mean ages were 45 year. Patients were excluded if they have infection, cardiovascular, inflammatory, neoplastic, hepatic or renal disorders rather than DN. In addition, there were 19 healthy age and sex matched subjects who served as control group. Consent for the study was obtained from the ethical committee and informed consent obtained from patients and controls. Studied patients were divided according to their urine albumin/creatinine ratio (UACR) into three groups;

normoalbuminuria group (UACR<30mg/g), microalbuminuria group (UACR 30–300mg/g) and macroalbuminuria group (UACR>300mg/g) as follows: **Group a (Normoalbuminuric):** 24 diabetic patients. **Group b (Microalbuminuric):** 22 diabetic patients. **Group c (Macroalbuminuric):** 24 diabetic patients.

The studied patients were subjected to careful history taking, clinical examination and laboratory investigations including fasting, 2h post prandial blood glucose, HbA1c, serum cholesterol and serum triglycerides, serum creatinine, urinary albumin, and urinary creatinine. Urinary albumin creatinine ratio was calculated in a first-morning spot urine collection. Urine examination was done by dipsticks using Meditest comp 3 Macherey-NAGEL EURL.BP.135, 67722 France. The strip has 3 levels of color intensity corresponding to the level of protein in urine (30, 100, 300). We used it as a primary rough method for detection of normoalbuminurea, microalbuminurea and macralbuminurea for patients' classification then urinary protein level is accurately determined by albumin/creatinine ratio using cobas c311 auto analyzer commercial kits supplied by Roche Diagnostics which automatically calculate the albumin/creatinine ratio in each urine sample. Quantitative determination of urinary albumin was done by immunoturbidimetric assay, using ALBT2, Tina-quant Albumin Gen2 kit supplied by Roche Diagnostics. Estimation of (KIM-1) and (NGAL) were done by using ELISA Kit supplied Glory Diagnostics Del Rio, TX 78840, USA. Estimated glomerular filtration rate (e-GFR) was calculated using **Cockcroft-Gault GFR**⁽¹¹⁾ = (140-age) X (Wt in kg) X (0.85 if female) / (72 X Cr). Wt=weight, Cr= serum creatinine in mg/dL.⁽¹⁰⁾

Results were collected, tabulated, and statistically analyzed using statistical package SPSS version 10 (Chicago, USA). Two types of statistics were performed: Descriptive statistics - for example, mean (X) and SD - and analytic statistics. The 't' value was calculated and the probability (P) value then deduced. Mann-Whitney U-test (nonparametric test) is used for comparison between two groups not normally distributed having quantitative

variables. Analysis of variance (ANOVA) test is used to assess the statistical significance difference between more than 2 study group means. Receiver operating characteristic-curve (ROC-curve) is used to assess the diagnostic performance of the studied parameter for discriminating patients from controls. In addition, Spearman correlation coefficient (r) (nonparametric test) is used to measure the association between two quantitative variables. The level of significance was set as P value less than 0.05.

Results:-

There were no significant difference between control group and diabetic groups regarding age, blood pressure and serum urea but there were statistically significant difference regarding the rest of data.

The pair wise comparison between normoalbuminuric group and microalbuminuric group showed statistically significant difference in disease duration, u.albumin and NGAL but there were no statistically significant difference regarding the rest of data. The pair wise comparison between microalbuminuric and macroalbuminuric group showed statistically significant difference regarding u.albumin, albumin/creatinine ratio, cholesterol, KIM-1, NGAL, eGFR, serum creatinine, triglycerides and disease duration. The pair wise comparison between macroalbuminuric and normoalbuminuric groups showed statistically significant difference regarding disease duration, total cholesterol level, triglycerides, serum creatinine, u.albumin, UACR, uKIM-1, NGAL and eGFR (**Table 1**).

There were highly significant statistical positive correlations between uNGAL, serum cholesterol, triglycerides, HbA1c, disease duration. However, there were no significant statistical correlation regarding serum creatinine and eGFR. Correlation analysis between uKIM-1 and cholesterol, HbA1c and disease duration in diabetic patients revealed highly significant positive correlation. On the other hand there was a significant negative correlation between uKIM-1 and eGFR, but no significant statistical correlation regarding serum creatinine. Also there were highly significant positive correlation between u.albumin and cholesterol, disease duration, s.creatinine and triglycerides. On the other hand there is a significant negative correlation between u.albumin and eGFR, but no significant statistical correlation regarding HbA1c (**Table 2**).

There were highly significant positive correlation between u.albumin with both KIM-1 and NGAL (**Table 3**).

At the cutoff value 88.5 ng/L, the diagnostic sensitivity of KIM-1 was 91.6%, diagnostic specificity was 84%, at cutoff value of 3.25 the diagnostic sensitivity of NGAL $\mu\text{g/L}$ was 100%, diagnostic specificity was 89.4%. U.albumin had no cutoff point to differentiate the normoalbuminuric group from control group (**Table 4**) (**Fig. 1**).

At the cutoff value 91ng/L, the diagnostic sensitivity of KIM-1 was 100%, diagnostic specificity was 94.7%, at cutoff value 4.00 the diagnostic sensitivity of NGAL $\mu\text{g/L}$ was 100%, diagnostic specificity was 100%. While at the cutoff value 19.4mg/dl, the diagnostic sensitivity for u.albumin was 95.4%, diagnostic specificity was 89.4% to differentiate the microalbuminuric from control group (**Table 5**) (**Fig. 2**).

Discussion:-

Despite increasing knowledge on prevention and treatment of diabetic kidney disease, it is still a significant cause of end-stage renal disease with high morbidity and mortality. Tubular impairment plays a pivotal role in the pathogenesis of diabetic kidney disease. ⁽¹²⁾ High glucose levels and diabetic substrates, including advanced glycation end-products, carbonyl intermediates, and growth factors, promote renal tubular hypertrophy and fibrosis. ⁽¹³⁾ Therefore, tubular biomarkers may be crucial as glomerular markers for early diagnosis of renal impairment in T2DM. The diagnosis and follow up of renal dysfunction in diabetic patients is commonly based on urinary albumin excretion and glomerular filtration rate. However estimations of GFR reflect late functional changes and not early structural abnormalities in the kidney and is creatinine based which is affected by many factors, including muscle mass, age, gender and race. ⁽¹⁴⁾ Micro albuminuria, as a reliable marker for early diabetic nephropathy has many argument related to the effect of exercise, urinary tract infection, acute illness and heart failure. ⁽¹⁵⁾ Renal impairment occurs in normoalbuminuric state or before the onset of microalbuminuria and can occur in non-diabetics, signifying lack of sensitivity and specificity of albuminuria for early detection of diabetic nephropathy. ⁽¹⁶⁾ New sensitive and specific markers in addition to albuminuria are needed to identify early renal tubulointerstitial damage in diabetic patients. Among these NGAL and KIM-1 are founded. We included in this study diabetic normoalbuminuric patients as well as patients with different stages of albuminuria compared to healthy control subjects with the aim to evaluate both urinary neutrophil gelatinase-associated lipocalin (NGAL) and urinary kidney injury molecule-1 (KIM-1) as early markers of renal dysfunction in type2 diabetic Egyptian patients. In this study, fasting blood glucose and HbA1c levels in the microalbuminuric and macroalbuminuric diabetic group were

significantly higher compared to diabetic normoalbuminuric and control groups. These results are in accordance with the other studies which reported that hyperglycemia is the driving force for the development of DN. ⁽¹²⁾ Elevated HbA1c is associated with the development of microangiopathy because HbA1c has a special affinity for oxygen, causing tissue anoxia and microangiopathy. ⁽¹⁷⁾ We found an increased NGAL level in normoalbuminuric subgroup of diabetic patients compared to the control group and its urinary excretion had a statistically significant positive correlation with the degree of albuminuria in micro-macroalbuminuric patients. This finding was similar to what is observed by **Zachwieja et al** ⁽¹⁸⁾ after studying the level of serum and urine NGAL in children with normal-range albuminuria, they suggest that normal-range albuminuria could not exclude DN. Thus, the increased urinary NGAL levels may reflect the degree of subclinical tubular impairment, representing an earlier measurable marker of renal injury. ⁽¹⁹⁾ In contrast to **Assal's** ⁽²⁰⁾ study who found a significant correlation between urine NGAL and serum creatinine, our study was in line with **Zachwieja et al** ⁽¹⁸⁾ showed no statistically significant correlation with serum creatinine and eGFR. This can be referred to that our study excluded severe renal impaired patients. **Wu et al** ⁽²¹⁾ reported markedly increased uNGAL in type2 diabetic patients from the normoalbuminuria to the macroalbuminuria and urinary NGAL showed strong positive correlations with urinary albumin creatinine ratio (ACR) and negative correlation with eGFR. In agreement with this study, **Thrailkill et al** ⁽²²⁾ reported a significant positive correlation between uNGAL and duration of the disease. Other authors reported the absence of this correlation. ⁽²³⁾ KIM-1 is not detected in normal kidneys but it is expressed in surviving renal proximal tubules after injury and can be quantified in the urine. So, uKIM-1 could be a sensitive marker for nephropathy. ⁽²⁴⁾ Our study showed a statistical significant increase of KIM-1 in macroalbuminuric group than microalbuminuric group but we didn't find a significant difference between normoalbuminuric group and microalbuminuric group. These findings suggest that KIM-1 may cause kidney injury in mechanisms that differ from albuminuria. **Peralta et al** ⁽²⁵⁾ concluded that KIM-1 is associated with increased risk of deterioration in renal function and incident chronic kidney disease independent of albuminuria. Previous studies revealed that expression of KIM-1 correlated positively with interstitial damage, inflammation, and serum creatinine, but did not correlate with proteinuria. ⁽²⁶⁾ In our study, there is a significant negative correlation between uKIM-1 and eGFR. But, there is no significant statistical correlation regarding serum creatinine. Urinary excretion of KIM-1 is not only related to the tubular production but also specific to tubular damage, as confirmed by biopsies in both clinical and experimental studies. Therefore, KIM-1 is a good marker of active tubular damage and not tubular scarring. ⁽²⁷⁾

Dyslipidemia is considered as a risk factor for diabetic nephropathy. We found a significant positive correlation between urine NGAL, KIM-1 and cholesterol in diabetic patients, though others could not find this correlation. ⁽²³⁾ In our study, the urinary albumin excretion in diabetic patients was linked to the duration of diabetes mellitus. These findings were in line with **Kondaveeti et al** ⁽²⁸⁾, who found direct correlation between the duration of diabetes and the development of microalbuminuria. Long-standing hyperglycemia promotes release of pro-inflammatory cytokine and expression of growth factor resulting in mesangial expansion and later fibrosis. ⁽²⁹⁾ There were highly significant positive correlation between u.albumin, serum cholesterol and triglycerides. There was no significant statistical Correlation between albumin and HbA1c, this in accordance with **Solakibrahim** ⁽³⁰⁾ who observed that no statistically significant relation between HbA1c and UAE levels during follow up of diabetic patients for 4 years. On the other hand there is a significant negative correlation between u.albumin and eGFR and significant positive correlation with serum creatinine. **Berhane and co-authors** ⁽³¹⁾ reported that a combined measure of albuminuria and eGFR is a significant predictor of end-stage renal disease. As our study included diabetic patients with mild diabetic nephropathy (eGFR>60), we expected that relatively less severe tubulointerstitial damage in our patients with early DN might lead to the failure of the tubular injury markers as NGAL to correlate with the GFR.

ROC curves were done to assess the diagnostic performance of uNGAL, uKIM-1 and u.albumin to differentiate normoalbuminuric and microalbuminuric cases from control. AUC-ROC analyses indicated better diagnostic performance of NGAL and KIM-1 with excellent diagnostic accuracy AUC (1.0-0.9), while u.albumin failed to discriminate the normoalbuminuric group from control group. In addition, uNGAL showed relatively better area under curve for estimating microalbuminuria, demonstrating its value as a sensitive marker for detecting the onset of DN.

In conclusion, both uKIM-1 and uNGAL are more sensitive and specific than u.albumin in early detection of diabetic nephropathy with higher diagnostic sensitivity and specificity to uNGAL than uKIM-1. The results reflect a single center cross sectional study in a limited number of patients. We have not confirmed our hypothesis of renal tubular damage with biopsy; however, clinical guidelines limit renal biopsy in conditions with an overt cause of proteinuria, such as diabetic nephropathy.

The authors declare no conflict of interest

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