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Evaluation of Serum Neutrophil Gelatinase Associated Lipocalin Level as an Early Biomarkers for Type 2 Diabetic Nephropathy

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Abstract

Background: Diabetic nephropathy is an emerging clinical and public health challenge and is related with adverse outcomes including ESRD and heart failure, as well as kidney replacement therapy , deaths related to these terminal illnesses . Diabetic kidney disease DKD and CVD are leading causes of morbidity and mortality in T2DM. The Neutrophil Gelatinase Associated Lipocalin (NGAL) is specifically released into blood and urine as a reaction to nephron injury. Higher levels of NGAL were reported in patients with diabetes in comparison to healthy nondiabetic individuals.

The aim of study: The study aims to evaluate the The study aims to evaluate the serum NGAL as biomarker for early detection of nephropathy in type 2 diabetic patients.**Patients & Methods:** Case control study was carried out in Salah-Addin city in private nephrologist clinic from 1t October 2020 to 1st February 2021.The study included conducted on 90 individuals , 60 diagnosed T2DM patients (30 had diabetic nephropathy which had albuminurea and 30 had diabetic without nephropathy had normal urine albumin) and the remaining 30 who participated as a control group. Blood samples were collected from each patients and controls to evaluate the levels of NGAL. All data were analyzed by a computer using SPSS program.**Results:**The study showed significant difference in serum NGAL (mean±SE) (1131.00±84.88 pg\ml) . The Pearson correlation test showed that there was non significant negative Correlation of serum NGAL with age and with serum creatinine.**Conclusions:** The results of the current study showed a significant increase in serum NGAL level of DN patients with T2DM and may be

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considers a good biomarker for prediction in the case of diabetic nephropathy.

Keywords: Diabetic nephropathy; Type 2 Diabetes Mellitus; Neutrophil Gelatinase Associated Lipocalin.

Introduction

Diabetes mellitus (DM) is a common and serious metabolic condition which presents a major challenge in twenty-first-century healthcare. The global prevalence of DM has dramatically increased from 108 million in 1980 to 451 million in 2017 and this number continues to rapidly rise [(1)]. From a medical perspective, it represents a series of metabolic conditions associated with hyperglycaemia and caused by partial or total insulin insufficiency [(2,3)]. The most prevalent form of diabetes by far is T2DM , affecting about 90 % of people with diabetes, while the remaining 10 % mainly have T1DM or gestational diabetes [(4)] . It can lead to progressive development of multidimensional complications as to vascular system of human body . Complications of micro-vascular endpoints may include retinopathy, nephropathy and neuropathy, While Cardiovascular disorders, heart failure, atherosclerosis and cerebrovascular events primarily result from damage to the macrovasculature [(5,6)]. Diabetic nephropathy is an emerging clinical and public health challenge and is related with adverse outcomes including ESRD and heart failure, as well as kidney replacement therapy , deaths related to these terminal illnesses [(7)]. Diabetic kidney disease DKD and CVD are leading causes of morbidity and mortality in T2DM [(8)] . The terms Diabetic nephropathy and DKD are used interchangeably to describe a set of characteristic clinical and pathologic findings. The main clinical findings of DKD are the presence of albuminuria, progressive CKD, and less commonly microscopic hematuria. Diabetes is the most common cause of the nephrotic syndrome. Proteinuria is first detected by screening for albumin in the urine [(9)]. Various factors may be associated with a worsening renal disease among diabetic patients. Some of the major factors include genetic predisposition , improper control of blood sugar and hypertension [(7)]. Growing evidence supports an integral role of tubular and interstitial compartments damage in the pathogenesis of DKD, which correlates well with progressive renal function decline and represents a final common pathway of CKD[(10)]. Neutrophil gelatinase-associated lipocalin is expressed in very minimal levels in

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numerous pathological and experimental-induced conditions in many organs and tissues such as liver, kidney, lungs, bone, brain, hearts, stomach, and colon [(11)] . Neutrophil gelatinase-associated lipocalin has been recognized as one of the most promising biomarkers of the early stages of kidney damage in DKD [(10)]. Tubulointerstitial injury plays an important role in DN development process and even prior to glomerular injury. In addition, about one third of the patients with diabetes mellitus have decreased renal function prior to proteinuria[(12)]. In physiological process, recent researches have proved that NGAL can trigger nephrogenesis by stimulating the conversion of mesenchymal cells into kidney epithelia. Serum NGAL increases in the very early stage of diabetic nephropathy and drops down as the disease develops[(11)]. we should pay more attention to the biomarkers of tubulointerstitial injury such as NGAL , which can contribute to the early diagnosis and treatment of DN patients[(12)]. Neutrophil gelatinase-associated lipocalin is a secreted protein and can be quantified in plasma or serum. Neutrophil gelatinase-associated lipocalin levels analysis is also possible in urine . These elements, combined with its good stability and resistance to proteases, make it a biomarker of choice for clinical use[(13)] .

Patients and Method

case control study conducted on 90 individuals , 60 diagnosed T2DM patients (30 had diabetic nephropathy which had albuminurea and 30 had diabetic without nephropathy had normal urine albumin) and the remaining 30 who participated as a control group. The study carried out in Salah al-Din Governorate Tikrit City, a private nephrologist clinic from 1t Octoberr,2020 to 1st February,2021. The information about patients in this study was retrieved from patient's itself. Their mean ages were(51.07±1.35) years and mean BMI (mean±SE) (25.83±0.38) . The criteria of exclusion include those diagnosed as T1DM , kidney disease other than DN , Patients with a history of usage of renin–angiotensin system inhibitors , history of usage of nonsteroidal anti-inflammatory drug (NSAID), nephrotoxic medications, or immunosuppressant , pregnancy were also excluded from the study.The diabetic patients (with and without) nephropathy were diagnosed by analysis Random blood glucose , HbA1c and presense or absense albuminurea. About five milliliters of blood were collected from the antecubital vein of

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patients and controls in plain tubes without any anticoagulant at room temperature for 10-15 minutes and allowed to clot. The tube then were centrifuged (3000 rpm) for 15min. The clear serum was pipetted into clear dry Eppendorf's and stored at (-20oC) until used for the various investigations (NGAL ,RBS, Blood urea, Creatinine and HbA1c%). The levels of NGAL was measured by using Enzyme-linked immunosorbent assay (ELISA). About 10 milliliters of urine were collected to test Albuminuria which tested by dipstick methodBody mass index (BMI) and Glomerular filtration rate (eGFR) were calculated. All data were analyzed by a computer using SPSS program

Results

The study showed there was significant difference level in DN patients compared to diabetic patients without nephropathy, and healthy control group concerning NGAL level. The highest mean of NGAL(mean±SE) (1131.00±84.88 pg\ml) was occurred in DN patients. as shown in the Table 1.

Table 4-1: Level of Neutrophil Gelatinase-Associated Lipocalin in Diabetic Nephropathy and Diabetic Without Nephropathy Patients and Control Group.

	NGAL(pg\ml)		
Groups	D& N	D	Cont
No.	30	30	30
Mean	1131.00	388.74	124.94
SE	84.88	68.02	2.92
P . value	0.01		
	0.01		

Pearson's correlation test reveals that there were non significant negative Correlation between NGAL with age(r = - 0.14) and serum creatinine (r = - 0.14) in DN patients As showen in figures(1,4) . While our findings showed there was no correlation with BMI (r

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= - 0.08), duration of disease(r = - 0.06), blood urea(r = - 0.009) and eGFR (r = - 0.09). As showen in figures(2,3,5and 6)

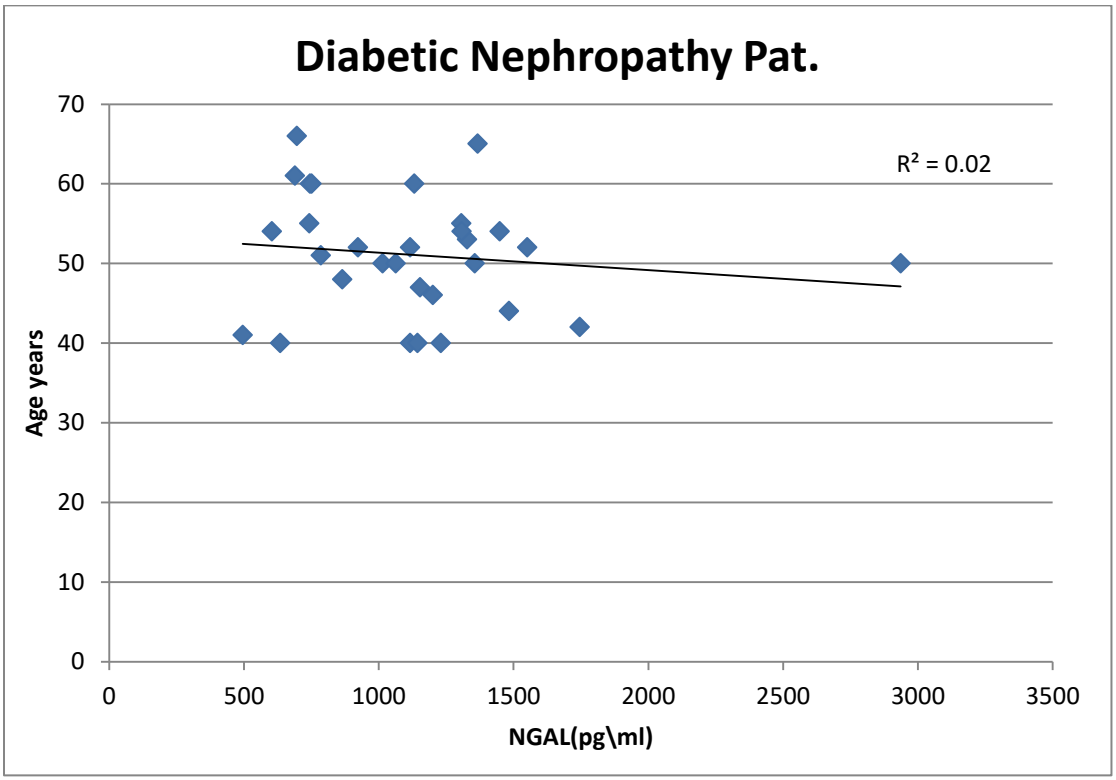

Figure 1: Correlation Between NGAL and Age in DN Patients

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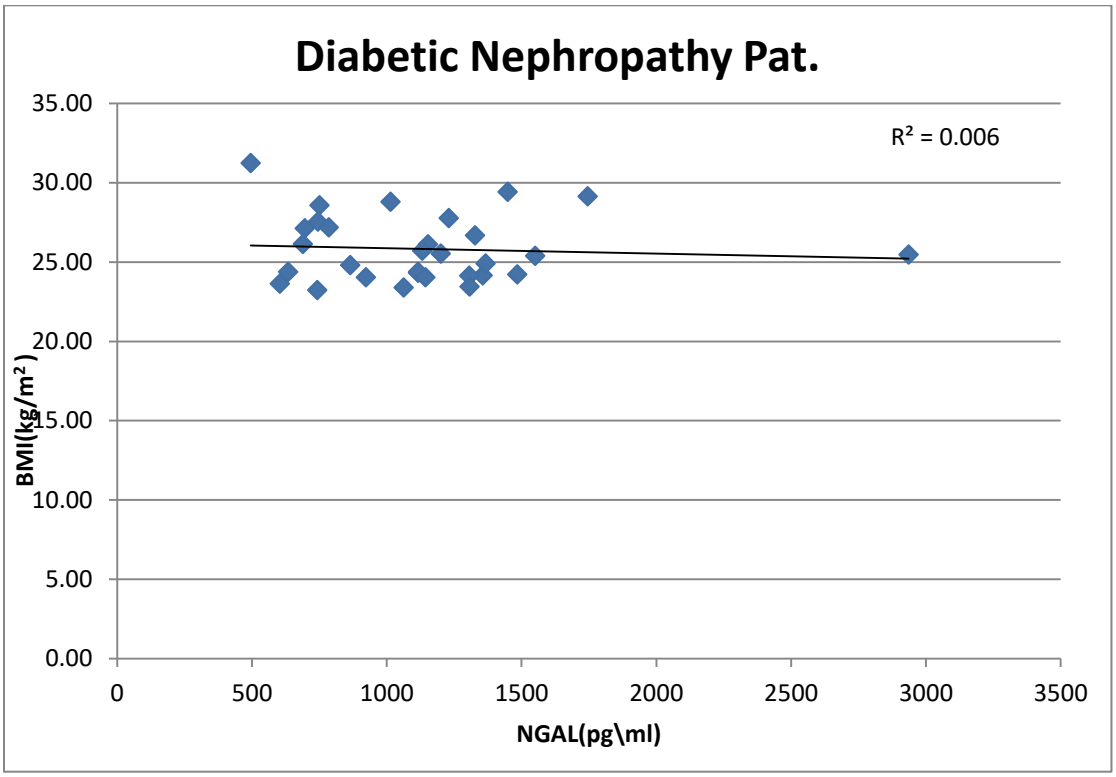

Figure 2 : Correlation Between NGAL and BMI in DN Patients

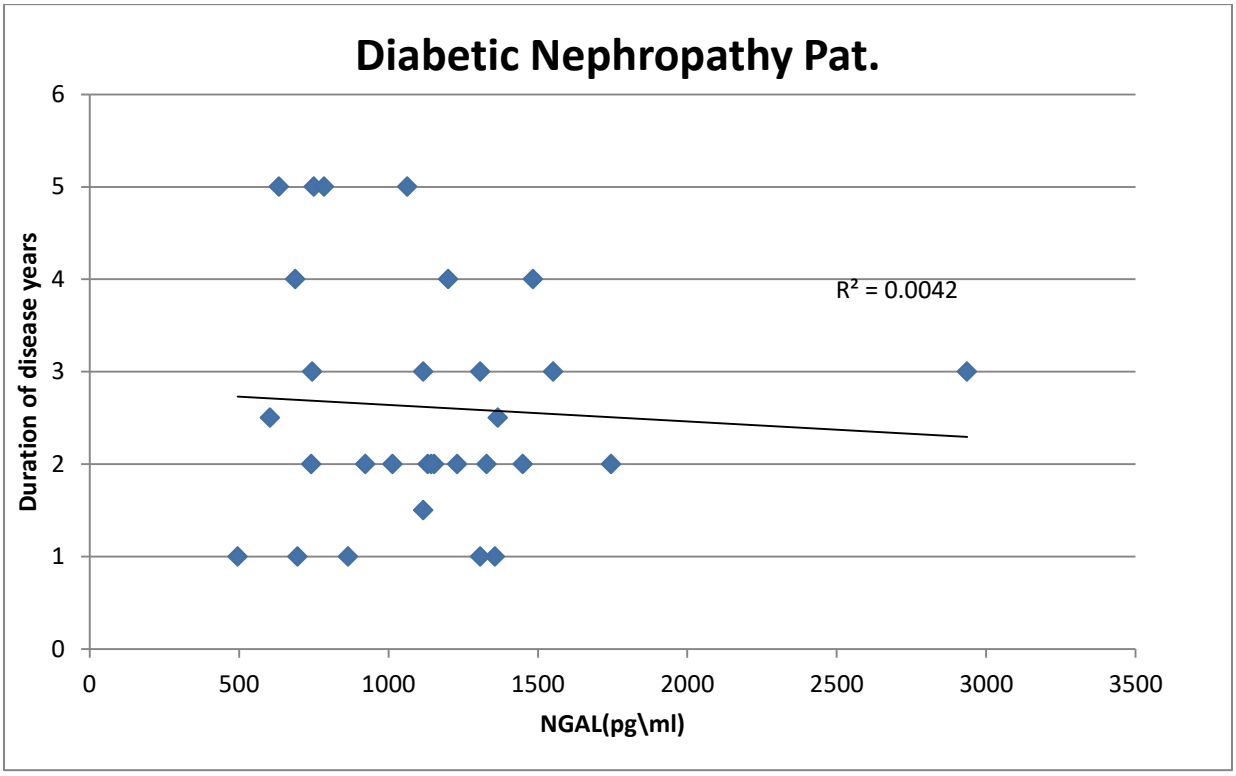

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Figure 3 : Correlation Between NGAL and Duration of Disease in DN Patients.

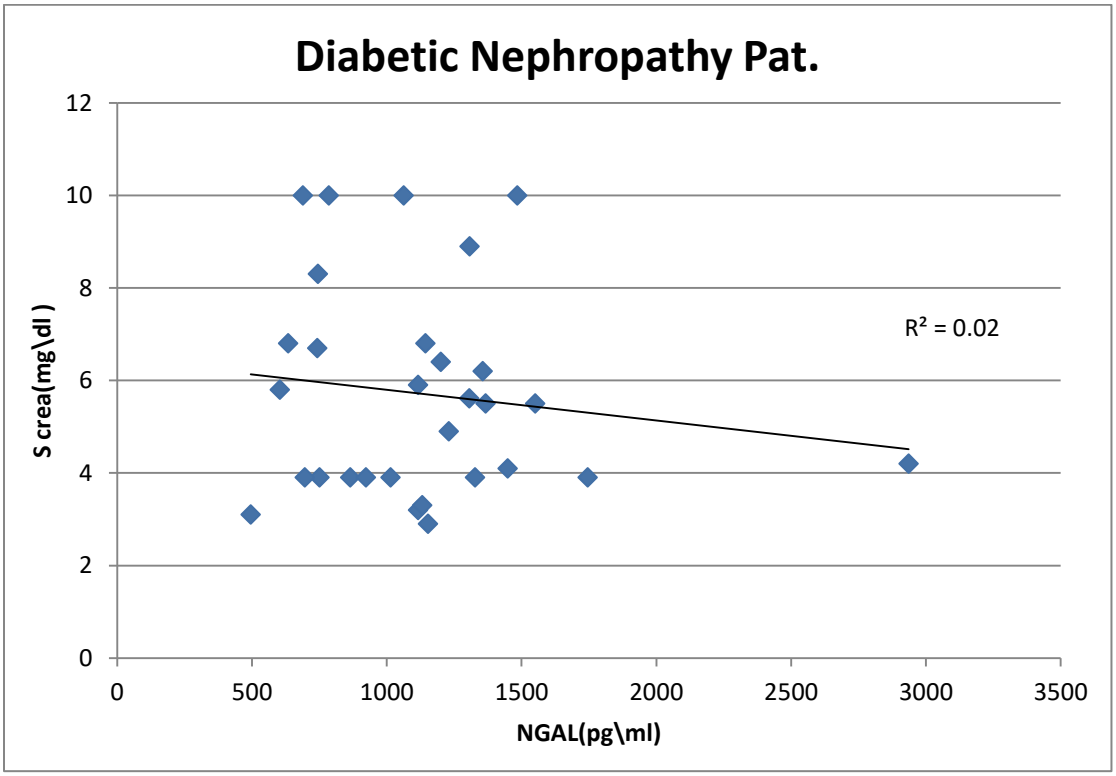

Figure 4-4 : Correlation Between NGAL and Serum Creatinine in DN Patients.

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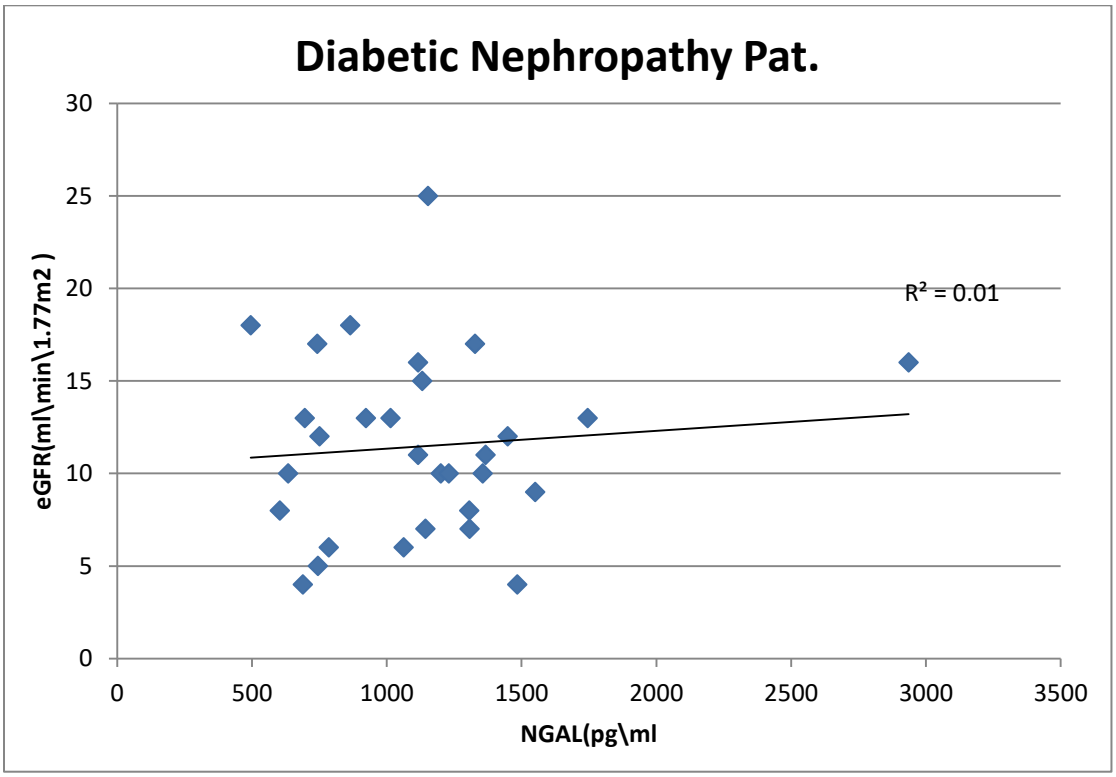

Figure 4-6 : Correlation Between NGAL and eGFR in DN Patients.

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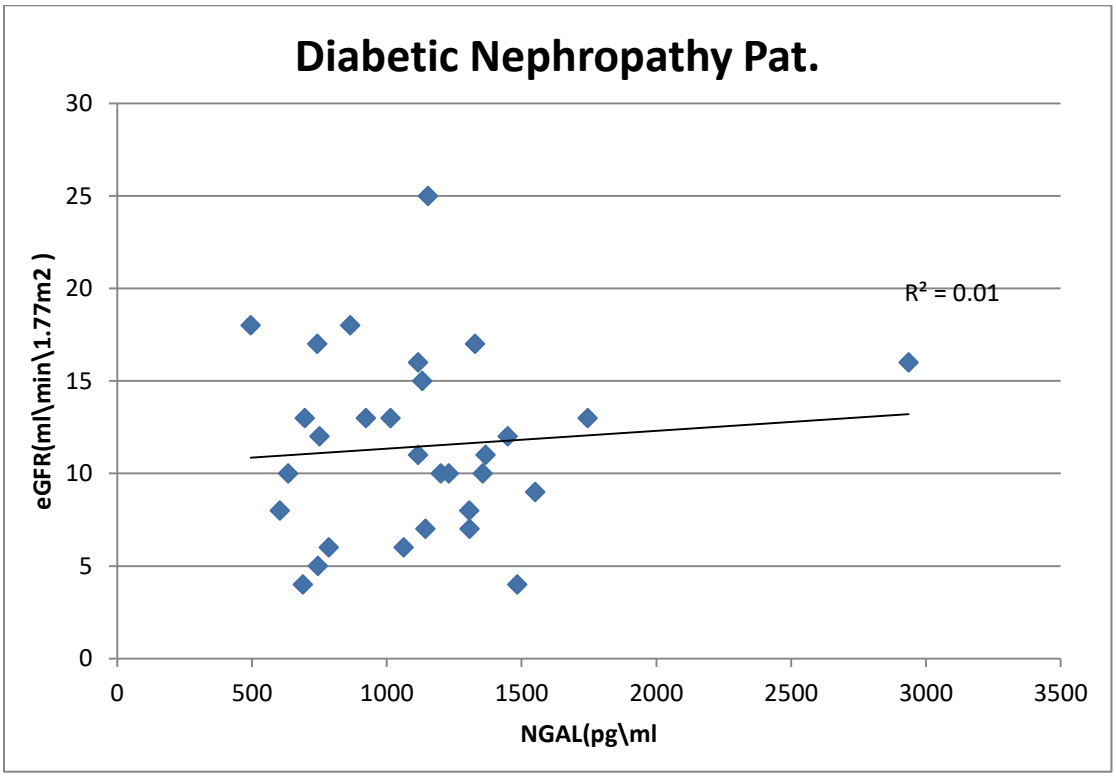

Figure 4-6 : Correlation Between NGAL and eGFR in DN Patients.

Discussion

Diabetic nephropathy is one of the most common complications of T2DM and a strong predictor of end-stage renal disease in these patients[14]. The pathophysiologic changes in DN linked to renal function decline are associated with cellular and extracellular derangements in both the glomerular and tubular compartments[15]. Standard biomarkers including serum creatinine, estimated glomerular filtration rate and albuminurea are imprecise, do not directly measure renal tissue injury, and are relatively insensitive to small changes in renal function. So, there is a need for another biomarkers that might provide a sensitive and fast means for identification of the progression of DN [16,14,17]. Early diagnosis of DN is difficult due to the fact that up to 50% of kidney function can be lost even before creatinine rises. In this study, the levels of NGAL,, as well as its relationships with various study parameters, were measured in DN patients. This approach is crucial for deciding whether these parameters can be used as early biomarkers of DN daigonosis.Results presented in (Table 1) show increased in the

NGAL (pg / ml) level in DN patients compared to diabetic patients without nephropathy, and healthy control group. The mean value of serum (NGAL) was significantly higher in DN than to diabetic patients without nephropathy and healthy control group ($p = 0.01$). In agreement with our findings in studying the elevation of t serum NGAL level is significantly increased in DN patients , DyabAllawi *et al* [18] and Sueud *et al* [19], The results of these studies showed that serum NGAL level is significantly increased in diabetic patients with microalbuminuria compared to diabetic patients with normal albuminuria and control group. Similarly, Motawi *et al* [20] Mahfouz *et al* [21] , registered serum NGAL was significantly higher in T2DM with microalbuminuria group compared to T2DM with normalbuminuria and control groups with significant positive correlation with age and duration of DM. The findings of Abbasi *et al* [22] suggest that urinary NGAL levels increase before the onset of traditional markers (eGFR and albuminuria) in T2D patients , which indicates that urinary NGAL could be a complementary measurement in the early diagnosis of diabetic nephropathy .Kapoula *et al* [23] also agreed with our current findings and summarized, NGAL values, both serum and urine, showed an increased trend achieving the highest concentrations in patients with the highest degree of severity of DN. Kaul *et al* [24] and Vijay *et al* [25] also agreed with our current findings and reported that NGAL level was significantly higher in diabetic individuals with albuminuria as comparing with healthy control. The study was disagreed with study done earlier by Sharif *et al* [11] on Urine samples of 123 patients were acquired from the Qatar Biobank the results of this study showed no significant difference was found in mean values of NGAL concentrations in healthy patients, diabetic patients with HbA1c>6% and diabetic patients with HbA1c<6% ($p>0.05$). Despite the lack of an adequate explanation, authors suggested that serum NGAL may increase in the very early stage of diabetic nephropathy and drop down as the disease develops; as the disease progresses, excretion of NGAL in urine increases and the absorption function of tubule decreases. [26]. The results showed that non significant negative Correlation between NGAL with age($r = - 0.14$) and serum creatinine ($r = - 0.14$) in DN patients. In agreement with the current study Sueud *et al* [19] showed that was NGAL negatively correlated with the age in diabetic patients with nephropathy. Also our finding results agreed with the

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El-ijla *et al* [27] found that uNGAL express a statistically significant negative correlation with creatinine (r = -0.273, p = 0.018). In the other hand unlike our results a study done by Quang *et al* [28] and Bennett *et al* [29] registered there was a positive Correlation between NGAL and age. Bolignano *et al*[30] and Papadopoulou[31] who found that NGAL was positively correlated with creatinine. Hafez *et al* [32] and Nielsen *et al* [33] were found that there was no association between NGAL and age. Unlike our results studies done by Lacquaniti *et al*[34] and Zachwieja *et al*[35] they found no correlation NGAL and Serum Creatinine. our findings showed there was no correlation with BMI (r = - 0.08), duration of disease(r = - 0.06), blood urea(r = - 0.009) and eGFR (r = - 0.09).Studies agreed with Our finding results Sharif *et al* [11], El-ijla *et al* [27] observed that NGAL has no correlation with BMI, duration of disease and eGFR . Our finding results also agreed with the study done by Quang *et al* [28] and Machado *et al* [36] who reported that there was no Correlation between NGAL and blood urea. In the other hand unlike our results a study done by Sueud *et al* [19] showed that was NGAL negatively correlated with the BMI. While study done by Na *et al* [37] found that serum NGAL levels were positively correlated with BMI. Also in contrast to our study results studies done by DyabAllawi *et al* [18], Mahfouz *et al* [21] they found statistical positive association was found between NGAL and duration of the disease while showed that there was negative correlation between serum NGAL and eGFR. The variation in findings may be due to sample size, type of diabetes, type of kidney injury, stage of disease ,or ethnicity[11] .

Conclusion

In comparison to diabetic patients without nephropathy and a healthy control group, the serum level of Neutrophil Gelatinase-Associated Lipocalin increased in DN patients. NGAL may be used as a predictive marker for the early detection of DN in patients with T2DM.

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