

Evaluation of Haptoglobin and Neutrophil Gelatinase Associated Lipocalin in Iraqi Patient with Type 2 Diabetic Nephropathy

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Abstract

Diabetic Nephropathy (DN) is referred as the kidney injury that occurs in individuals with diabetes, the earliest detectable change is the thickening in the glomerulus. The study objectives to estimate the diagnostic value plasma levels of Haptoglobin (HPt) and neutrophil gelatinase associated lipocalin (NGAL) as biomarkers for early disclosure of nephropathy in type 2 diabetic (T2D) patients. The present study was conducted on sixty T2D patients diagnosed beforehand, these patients were categorized into two equal groups according to their albumin/ creatinine ratio (ACR), including patients with normoalbuminuria (I) (ACR<30mg/g Creatinine) and patients with microalbuminuria (II) (ACR=30-300 mg/g creatinine), twenty four healthy were chosen as a control group (III). Aspartate aminotransferase (AST), alanine aminotransferase (ALT), hemoglobin (Hb), U.Mic, U.Cr, ACR, HPt and NGAL were measured in the studied groups. NGAL & HP were found to have a strong positive correlation with fasting plasma glucose (FPG), urine microalbuminuria (U.Mic), urine Creatinine (U.Cr) and (ACR). The present study reveals high significant differences between male and female diabetic patient groups in terms of Hb.

In conclusion, a significant role for plasma NGAL and HPt in pathogenesis and progression of renal harm in DM, and a strongly positive correlated with the severity of proteinuria. This may be clinically useful variables for predicting the improvement of DN in the early stages of T2DM in the Iraqi population. [DOI: [10.22401/JNUS.20.2.02](https://doi.org/10.22401/JNUS.20.2.02)]

Keywords: Diabetic Nephropathy, Neutrophil gelatinase associated lipocalin, Haptoglobin, Type 2 Diabetes Mellitus.

Introduction

Diabetic nephropathy (DN) is the most significant microangiopathy of the complexities of diabetes characterized by raised urinary albumin secretion ratio, hypertension, and the decrease in the renal assignment prompting end-stage renal disease. As well, these patients have a high peril of cardiovascular sickness. Which further increments with worsening kidney function.^[1] DN is a quickly rising problem in the evolving countries with the epidemic of type 2 diabetes mellitus (T2DM).^[2] Presently, variations in albuminuria are viewed as a sign of onset or advancement of DN. Diabetic patients agonize from progressive renal pathological variations and the decrease of kidney function improvement, even if urinary albumin levels are in the normal ranges, suggest that albuminuria is not the ideal marker for the primary exposure of DN.^[3]

Tubulointerstitial and glomerular damage assumes a part in the pathogenesis of DN, some tubular hurts significances have been investigated for early discovery.^[4] From them

is neutrophil gelatinase associated lipocalin (NGAL), which is created in epithelial cells and neutrophils.^[5] Former studies have demonstrated NGAL to be the most safely upregulated proteins in the renal tubules taking after ischemic harm, showing that it is a critical indicator for intense kidney damage.^[6] NGAL is a 25-kD a molecule known to be hyper manufactured in kidney tubules within a few hours after harming experimental stimuli. Tubular excretion of NGAL associates with the acuteness of chronic kidney failure, as in autosomal dominant polycystic renal disease or glomerulonephritis.^[7,8] Haptoglobin is a hemoglobin-binding protein existing in the plasma. Its function is principal to control the destiny of hemoglobin out from red platelets after either extravascular or intravascular hemolysis. There are two current genotype at the HPt genetic locus signified 1 and 2. There are functional variations between the HPt 1 and HPt 2 proteins produce in the preserve against hemoglobin-driven oxidative stress that seems to have paramount clinical significance. Specifically, individuals with the

HPt 2-2 genotypes and DM seem to be at safely greater peril of macrovascular and microvascular complexities. A strategy of managing high dose antioxidants, particularly to HPt 2-2 DM persons may be clinically active^[9] HPt is a plasma glycoprotein and a positive intense stage protein that joins free hemoglobin, forming hemoglobin-haptoglobin (Hb-HPt) stable complex Hb-HPt complex extracted by macrophage by means of the cell-surface receptor (CD163). HPt avoids tissue destruction caused by free hemoglobin and lessens iron loss in hemolytic situations. HPt doings as a lymphocyte assignment and modulated the helper T-cell type 1 and type 2 (Th1/Th2) balance within the body.^[10]

Patients and Methods

The present study was done in the National Diabetes Center for Treatment and Research at Al-Mustansiriya University between (February 2016-April 2016), our study included sixty T2D patients who were divided into two equal groups, group **I**, T2DM with norm albuminuria (albumin to Creatinine ratio [ACR] <30 mg/g creatinine) and group **II**, T2DM with microalbuminuria (ACR=30-300 mg/g creatinine). The two diabetic groups were described according to urinary albumin secretion, their ages ranged within (36-65) years for both genders. The initial stage in examination of DN is to measure of urinary albumin in urine specimen in the morning. Several variables were examined to be compared with twenty four healthy controls (group **III**), such as body max index (BMI), thoracic to hip ratio (T-H), waist to hip ratio (W-H), Sys and Dia BP is coordinating with the diabetic patients who register in the study. The Urine specimens were collected from each individual into a sterile container and utilized for the measurement of urine microalbuminuria (U.Mic), urine Creatinine (U.Cr) and ACR. Venous blood specimens were collected from diabetic patients and controls into two sorts of vacutainer tubes as follows: first venous blood sample was collected in plain tube and left to clot for (30min) at room temperature and then centrifuge to separate at (3000rpm) for (10min) utilized for the biochemical examines. Second tube with EDTA, mixed gently by the

shaker and then centrifuged to separate the plasma for 15min at 1000xg (2-8 c°) within 30 min of accumulation and then transferred to a new polypropylene tube, plasma samples were stored at -20°C until assayed. Fasting plasma glucose (FPG) level was determined according to a Trinder reaction by utilizing the glucose oxidase method, Cat. No. REF87409). Blood hemoglobin was measured according to the colorimetric method of drabkin's (Cat. No. REF1134015). ALT and AST levels were controlled by the utilizing of UV enzymatic technique (Cat. No. REF80027 and Cat. No. REF80025, respectively). Plasma NGAL and HPt were assessed using enzyme-linked Immunosorbent assay from the MyBiosource Company(Cat.No.#MBS584559,Cat.No.#MB702695, respectively).

Statistical Analysis

All information are expressed as means \pm SD, unless stated otherwise. In all cases, $P<0.05$ was viewed significant (two-tailed). The relationships between NGAL and HPt with other laboratory variables were analyzed by two-sided Pearson relationship test. Data were put away and processed by utilizing a commercially obtainable (SPSS for Windows, Version 20.0).

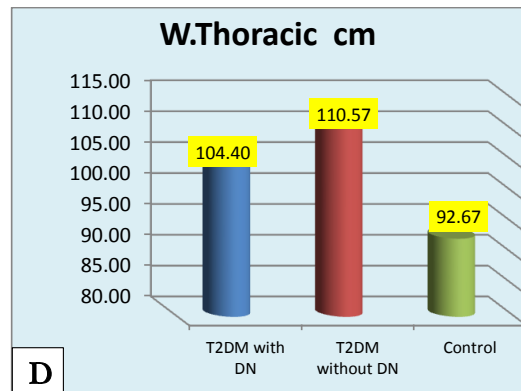
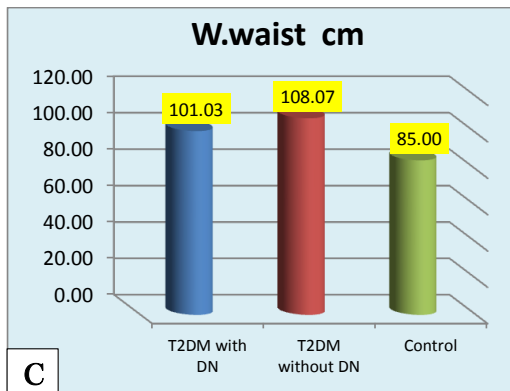
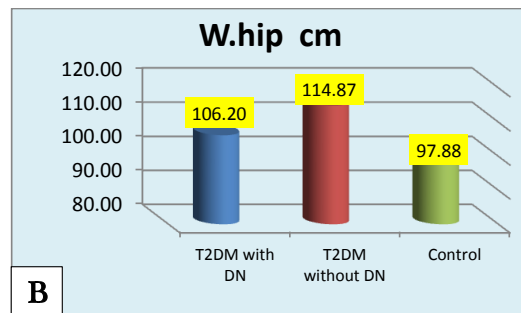
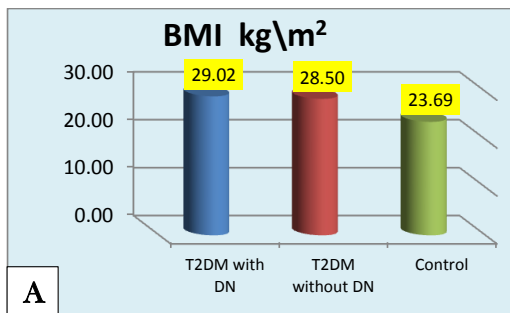
Results and Discussion

Our outcomes showed no statistically significant differences in term of age, group **I** & **II** showed a significant difference in BMI compared to group **III** ($P<0.05$) Fig.(1) (A). Also, there were statistically significant differences in the terms W. Hip, W, Waist & W. Thoracic between the normo and microalbuminuria diabetic groups (**I** & **II** groups) in comparison to group **III** as given in Table (1), Fig.(1) (B, C, & D respectively). The patients with norm-microalbuminuria group observed an important variation in the W-H ratio and W-T ratio. Higher Sys and Dia. BP levels were observed in **I** & **II** compared to group **III** Fig.(1) (E & F). The mean level of FPG was significantly higher ($P<0.05$) in diabetic groups as compared to the group **III** Fig.(1) (G).

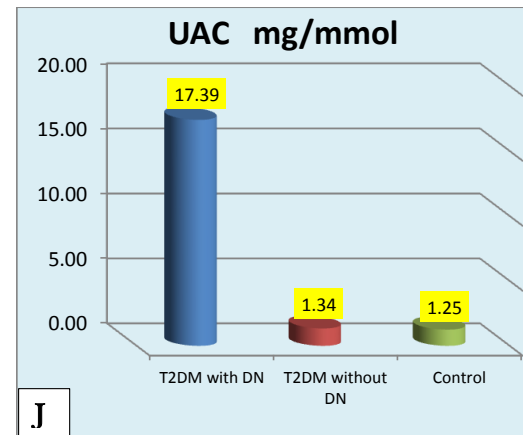
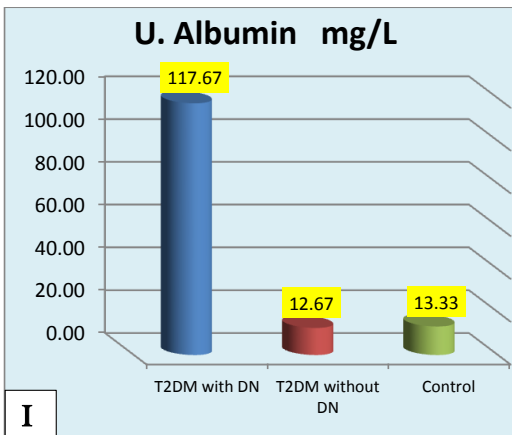
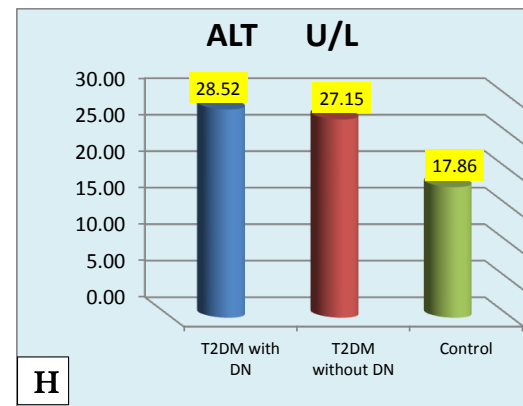
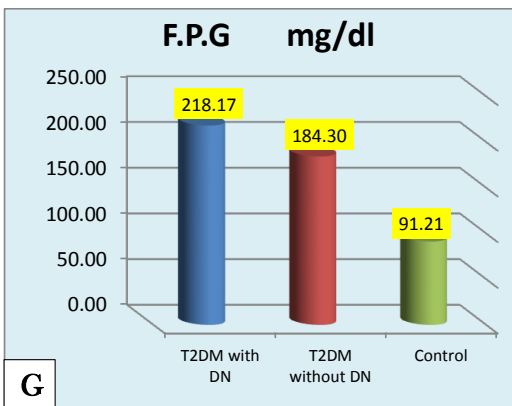
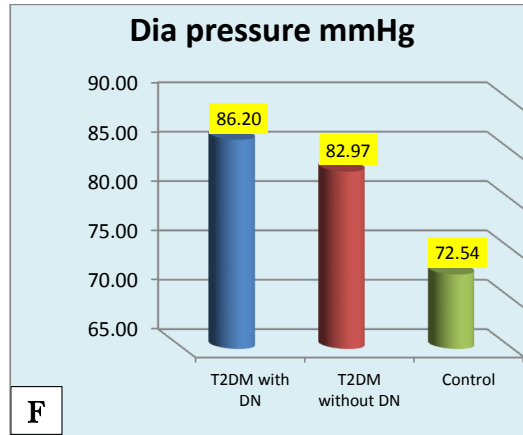
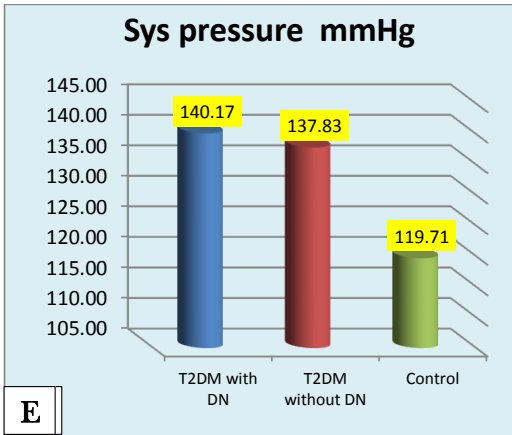
Table (1)
Mean and standard deviation for Various biochemical Variables in control, Case of DM with DN and Case of DM without DN.

Variables		Group I (Mean±SD)	Group II (Mean±SD)	Group III (Mean±SD)
Age	years	52.77±8.05 ^a	52.93±7.77 ^a	52.08±9.21 ^a
BMI	kg/m ²	28.50±3.82 ^a	29.02±3.75 ^a	23.69±2.29 ^b
W.hip	cm	114.87±13.60 ^b	106.20±14.38 ^a	97.88±9.80 ^c
W.waist	cm	108.07±9.88 ^a	101.03±17.66 ^a	85.00±11.04 ^b
W.thoracic	cm	110.57±12.47 ^b	104.40±11.24 ^a	92.67±8.78 ^c
W-H ratio	cm	108.07±9.88 ^a	101.03±17.66 ^a	85.00±11.04 ^b
W-T ratio	cm	110.57±12.47 ^b	104.40±11.24 ^a	92.67±8.78 ^c
SBP	mmHg	137.83±18.13 ^a	140.17±18.74 ^a	119.71±6.49 ^b
DBP	mmHg	82.97±8.00 ^a	86.20±11.59 ^a	72.54±5.33 ^b
Pulses	min ⁻¹	81.70±11.18 ^a	82.40±12.91 ^a	73.42±3.66 ^b
Hb	g/dl	13.09±1.30 ^a	14.20±4.52 ^a	12.89±1.30 ^a
FPG	mg/dl	184.30±42.85 ^b	218.17±65.10 ^a	91.21±7.64 ^c
ALT	U/l	27.15±11.71 ^a	28.52±16.81 ^a	17.86±4.63 ^b
AST	U/l	29.59±15.27 ^a	28.90±10.80 ^a	25.59±4.90 ^a
U. Mic.	mg/L	12.67±6.92 ^b	117.67±55.69 ^a	13.33±7.61 ^b
U. Cr	mmol/L	9.85±4.76 ^a	9.01±6.84 ^a	11.03±4.54 ^a
ACR	mg/mmol	1.34±0.05 ^b	17.39±9.95 ^a	1.25±0.44 ^b
NGAL	ng/ml	38.99±4.22 ^b	60.75±15.23 ^a	25.58±5.25 ^c
HPt	mg/dl	109.07±40.56 ^b	183.47±24.42 ^a	34.05±6.21 ^c

Notes: Similar letters mean no significant difference between the groups at a level of less than **0.05**, and various letters mean the existence of a significant difference at a level of less than **0.05**.



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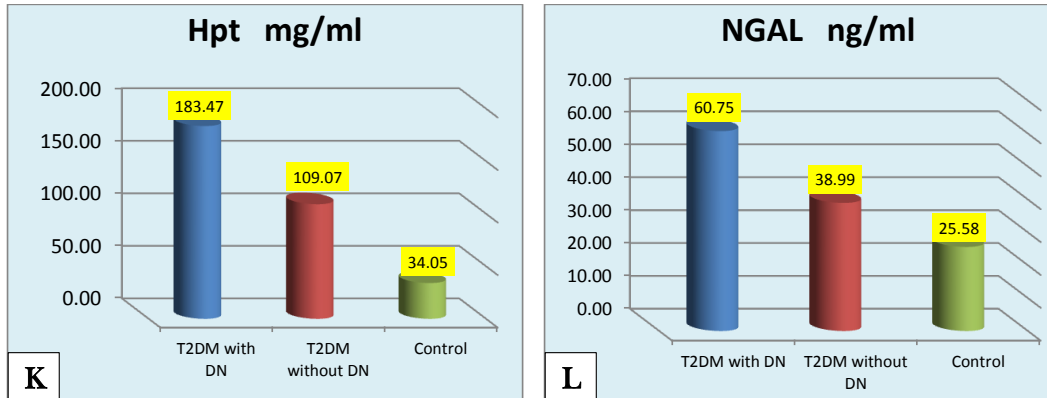


Fig.(1): Comparison between different in biochemical parameters studied groups.

Table (2)
Significant relationships between NGAL and HPT with other biochemical factors in diabetic patients with nephropathy.

Variables	R	P
NGAL-BMI	0.399	< 0.01
NGAL-Sys PB	0.412	< 0.01
NGAL-Dia PB	0.473	< 0.01
NGAL-Hb	0.315	< 0.01
NGAL-FPG	0.618	< 0.01
NGAL-U.Albumin	0.655	< 0.01
NGAL-UAC	0.638	< 0.01
HPT-BMI	0.420	< 0.01
HPT-Sys PB	0.444	< 0.01
HPT-Dia Pb	0.482	< 0.01
HPT-W.waist	0.323	< 0.05
HPT-ALT	0.326	< 0.01
HPT-FPG	0.631	< 0.01
HPT-U.Albumin	0.695	< 0.01
HPT-ACR	0.606	< 0.01

The statistical analysis of outcomes demonstrated that there no significant differences in Hb and U. Cr between the examined groups. Furthermore the ALT level was safely variations in diabetic groups than that in the control group, as shown in Table (1) Fig.(1) (H). Interestingly, the result shows that serum AST was not significantly different in the studied groups. Urinary-Mic and ACR were significantly increment in diabetic groups compared to group III and pronounced increment in the II group, Fig.(1) (I & J). Plasma concentration of NGAL and HPT were

significantly higher in patients with and without DN groups compared to a healthy group III, Fig.(1) (K & L), indicating that plasma NGAL and HPT are risk factors of microangiopathy in patients T2DM. There were significant positive relationship between plasma NGAL with BMI, Sys. PB, Dia. PB and Hb as shown in Table (2). The Pearson correlation test showed a strong positive correlation between plasma NGAL with FPG, U. albumin, UAC and plasma HPT, as shown in Table(2) Fig.(2) and (3). In additional, there were significant positive correlations between plasma HPT with BMI, Sys. PB, Dia. PB, W. Waist, and ALT and a strong positive correlation with FPG, U. albumin, ACR and plasma NGAL, as shown in Table (2) Fig.(4) and (5). The diabetic patients with normoalbuminuria showed a significant difference between male and female in terms of BMI, W. waist, W. thoracic, and pulses, interestingly, the diabetic patients without DN and control group demonstrated a significant difference between male and female in Hb, as appeared in Table (3).

Table (3)
The demographic and Anthropometric data of Diabetic Patients According to Gender.

Variables	Factor	Sex	Mean±SD	T. Test	Sig
BMI kg/m ²	Group I	M	29.51±4.04	2.003	0.055
		F	26.75±2.78		
	Group II	M	30.04±3.40	1.525	0.139
		F	28.00±3.92		
	Group III	M	22.49±2.20	-3.362	0.003
		F	25.12±1.48		
W. waist cm	Group I	M	111.16±8.92	2.436	0.021
		F	102.73±9.51		
	Group II	M	102.60±18.32	0.479	0.635
		F	99.47±17.48		
	Group III	M	82.62±11.90	-1.159	0.259
		F	87.82±9.71		
W. thoracic cm	Group I	M	114.00±13.69	2.095	0.045
		F	104.64±7.23		
	Group II	M	107.60±7.81	1.601	0.121
		F	101.20±13.37		
	Group III	M	90.31±10.14	-1.466	0.157
		F	95.45±6.17		
Pulses min ⁻¹	Group I	M	78.37±9.43	-2.298	0.029
		F	87.45±12.04		
	Group II	M	82.87±14.94	0.195	0.847
		F	81.93±11.03		
	Group III	M	73.31±5.75	-.155	0.878
		F	73.55±4.91		
Hb g/dl	Group I	M	13.64±1.23	2.176	0.038
		F	12.45±1.20		
	Group II	M	15.73±6.02	1.933	0.063
		F	12.68±1.01		
	Group III	M	13.62±1.14	3.715	0.001
		F	12.03±0.92		

In the present study, the level of FPG in the group **II** was significantly increment in comparison with **I** and **III** groups, these results are in agreement with the various studies, which have suggested that elevated blood glucose is the driving force for the advancement of DN. Hyperglycemia-stimulate promotes mitochondrial ROS generation as a consequence of glucose autoxidation metabolism and the formation of AGEs, ROS comprise the real source of intracellular ROS that outcome in the oxidative harm of proteins, lipid, and DNA, at last leading to apoptosis and kidney damage.^[11] DN is considered as an

inflammatory disease described by leukocyte infiltration at each phase of renal contribution.^[12] The high levels of U. Mic and ACR in diabetic patients with microalbuminuria group demonstrating that Microalbuminuria an early marker of DN, the elevated levels of urinary albumin excretion may represent a more generalized vascular harm than kidney microangiopathy damage alone.^[13] Microalbuminuria is linked with numerous of the disorders found in the insulin resistance disorder, including obesity and endothelial dysfunction in addition to type 2 diabetes.^[14]

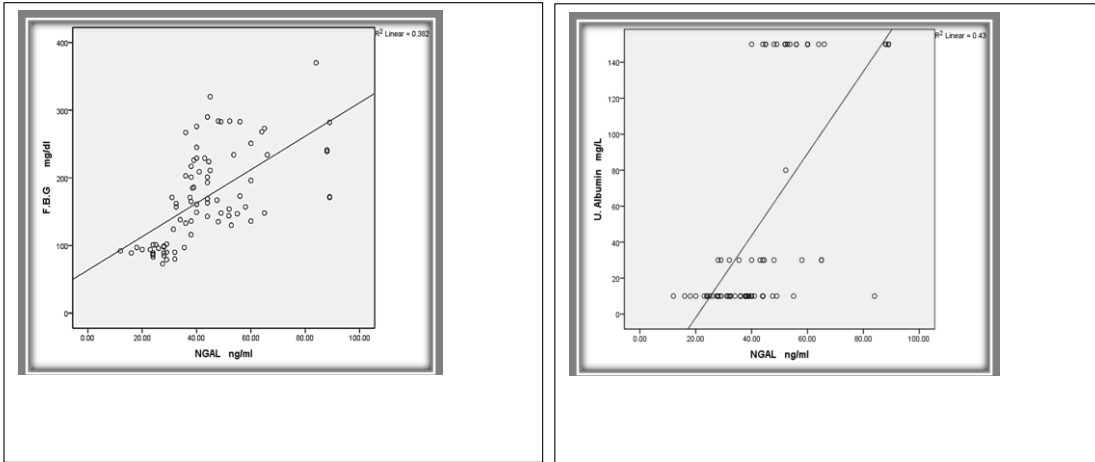


Fig.(2): The association between plasma NGAL with FPG and U. Albumin.

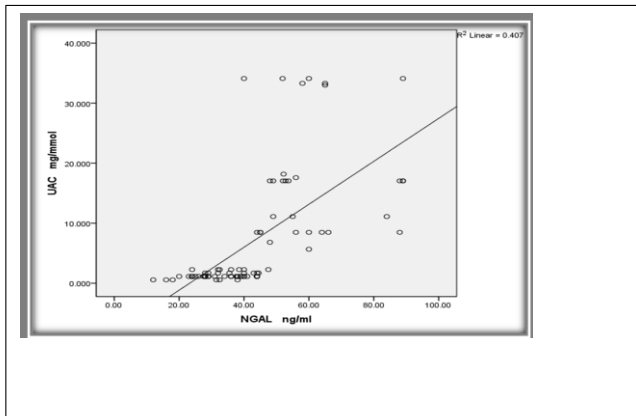


Fig.(3): The association between plasma NGAL with UAC.

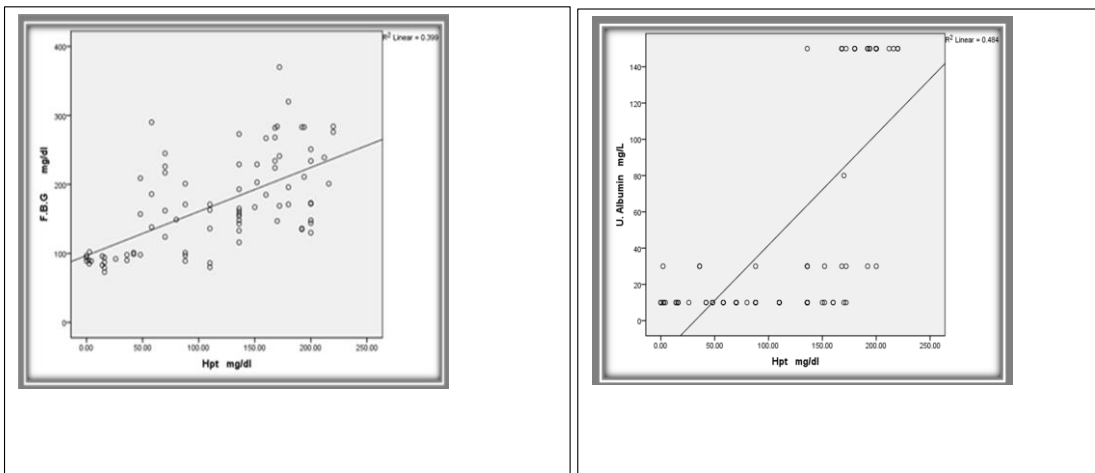


Fig.(4): The association between plasma Hpt with FPG and U. Albumin.

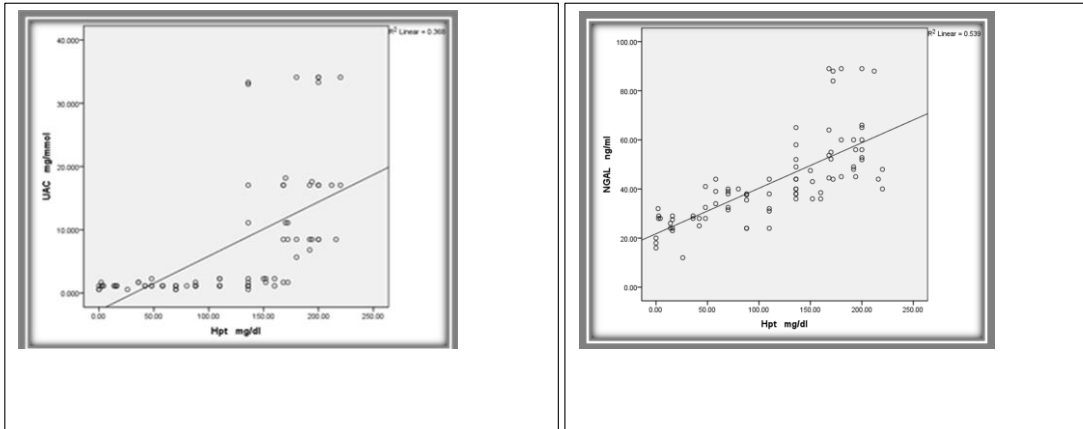


Fig.(5): The association between plasma HPT with UAC and NGAL.

Plasma NGAL was significantly higher in micro-normoalbuminuria diabetic groups compared to the control group. Recommending that lipocalin-2 might be an independent risk factor for hyperglycemia and insulin resistance in human. Several previous studies have proposed lipocalin-2 to be an intense -stage protein that can be initiated by a variety of inflammatory stimuli [15, 16] in type 2 diabetic patients, patients with microalbuminuria had higher plasma NGAL levels. This finding accept the hypothesis of an extensive increment in extra-renal creation of NGAL in diabetic patient eventually reduced clearance of NGAL because of decrease in renal function could also contribute to the high plasma NGAL levels particularly in the macroalbuminuria patients [17] NGAL plays a renoprotective part through upgrading tubule cell increase in kidney damage, particularly in ischemia-reperfusion harm, [18] an expanding body of evidence demonstrates that NGAL associates strongly with the kidney. NGAL can prompt the construction of renal epithelia by motivating the transformation of mesenchymal cells into proximal tubule epithelia, this is because NGAL can function as an iron-transporting protein to convey Fe^{2+} by forming a complex with iron-binding siderophores (NGAL: siderophore: Fe^{2+}) and Fe is essential for cell development and improvement. [19, 18] NGAL binding with large affinity to bacterial, iron binding proteins named siderophores. Iron, an especially ferrous shape is essential to bacteria for their development. In the case, very low levels of

free iron in the body make it a fundamental nutrient required by bacterial cells. [20]

Additionally, plasma NGAL a strong positively associated with FPG, U. Mic, and ACR. The Plasma NGAL level was elevated in diabetic patients with microalbuminuria compared to controls, indicating that tubular harm happens early and possibly before albumin secretion in patients with DN. [21] HPT a major increment in oxidative stress and oxidative tissue harm, particularly in the kidney in human. Free Hb in the circulation passes through the renal damage, encourages the generation of free radicals inside the kidney and glomerular filter and stimulates the generation of free radicals inside the kidney after the demolition of erythrocytes. [22] The quantity of hemoglobin penetrating blood vessel walls might be distinctly elevated in patients with DM. HPT can quickly bind free intravascular hemoglobin released into the circulation and stop hemoglobin-driven oxidation responses. [23] The outcomes of this study shows that a strong positive correlation was observed between plasma HPT concentration and FBG, Dia. BP & Sys. BP, intensive blood glucose levels near to the normal range has been demonstrated contributor to oxidative stress as accordingly elevated of HPT phenotype. Additional, expanded plasma HPT levels are real indications of adiposity in the individual. HPT is an intense phase protein that acts as an antioxidant by virtue of its ability to associate free Hb and avoid heme-iron interceded oxidation. [24] Hb is released into the

circulation, it binds immediately to HPt to form a HPt- Hb complex that is rapidly extracted predominately by the macrophage CD163 scavenger receptor expressed on Kupfer cells in the liver.^[25] There are expansive variation in body synthesis between man and women due sex steroid hormones levels between genders, the distinction of sex in mean hemoglobin level of vein is considered to be the important reason of the stimulatory impact of androgen in man's bone marrow, in addition to inhibiting influence of estrogen on woman's bone marrow.^[26] The mean red cell mass levels in the male are more than female due increased erythropoiesis by the impact of androgen in the male or the periodic loss of blood in female.^[27]

Conclusion

In conclusion, there were a strong positive correlation between plasma NGAL & HPt with FPG, U.albumin and ACR. The outcomes of the present study showed that plasma concentration of NGAL and HPt were significantly higher in patients with normo-microalbuminuria diabetic groups compared to a healthy control group, indicating that plasma NGAL & HPt may be good prognostic factors for renal dysfunction and the development of incipient nephropathy.

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