Serum Chemerin and Beta 2-Microglobulin in Type 2 Diabetes: Assessment of Diabetic Nephropathy

Aziza A Elsebai[1], Wessam E Saad[1] and Maram M Mahdy[2]

Departments of [1]Clinical and Chemical Pathology and [2]Internal Medicine, Faculty of Medicine, Ain Shams University
dr.wessamelsayed@gmail.com

Abstract: Background: Diabetic nephropathy is a serious microvascular complication of diabetes mellitus. Diabetic nephropathy is a clinical syndrome characterized by albuminuria, hypertension, and progressive renal insufficiency. Chemerin, a recently discovered adipocytokine, has been associated with autocrine/paracrine signaling for adipocyte differentiation and maturation .In addition ,it can regulate glucose uptake and stimulate lipolysis and is highly expressed in obese and insulin-resistant subjects and may play an important role in linking metabolic syndrome and inflammation. **Objective:** To study serum chemerin and beta2-microglobulin in diabetic patients with different stages of diabetic nephropathy, in a trial to explore their correlation with kidney functions and their value in assessment of disease severity. **Subjects &Methods**: The study included60adult patients with type 2 diabetes mellitus (Patients' group)and 40 age-and sex- matched healthy individuals serving as a control group.Type2 diabetic patients were subdivided according to urinary albumin excretion (UAE) into 3 subgroups, 1a (UAE<30 mg/24h) ,1b (UAE= >30-<300 mg/24h) and1C (UAE>300 mg/24h).All studied individuals were subjected to assessment of their fasting and postprandial blood glucose, UAE, serum creatinine, BUN, corrected creatinine clearance as well as serum chemerin and beta2 microglobulin measured by ELISA. **Results**: Serum chemerin and beta2microglobulin were significantly elevated among diabetic patients and a highly significant stepwise progressive increase in the marker level was recorded among patients subgroups. Serum chemerin and beta2 microglobulin were significantly higher in diabetic patients with macroalbuminuria[300(237.5-393.8 ng/mL),7.8(5.4-10.0µg/mL); respectively] than diabetic patients with microalbuminuria[155 (95-179.5 ng/mL),4.5(3.5-6.0µg/mL);p<0.01;respectively]compared to control [35(25-42.5ng/mL),2.0(1.5-2.35µg/mL);p<0.01;respectively]. Chemerin serum levels was significantly directly correlated with 2h- post prandial glucose (r_s=0.489, p<0.01) and fasting blood glucose(r_s = 0.405, p<0.05). Serum chemerin were significantly positively correlated with kidney functions as serum creatinine (r_s = 0.758, p<0.01), BUN(r_s = 0.643, p<0.01), serum beta 2-microglobulin (r_s = 0.818, p<0.01) and UAE (r_s = 0.975, p<0.01) and had a significant negative correlation with corrected creatinine clearance (r_s = - 0.769, p<0.01). **Conclusion:** Serum chemerin is a sensitive marker in assessment of diabetic nephropathy in type 2 diabetic patients, especially when combined with beta2-microglobulin.

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1.Introduction

Diabetic nephropathy, a long-term major microvascular complication of uncontrolled hyperglycemia, affects a large population worldwide. Recent findings suggest that numerous pathways are activated during the course of diabetes mellitus and that these pathways individually or collectively play a role in the induction and progression of diabetic nephropathy. However, clinical strategies targeting these pathways to manage diabetic nephropathy remain unsatisfactory, as the number of diabetic patients with nephropathy is increasing yearly[1].

Diabetic nephropathy has several distinct phases of development. Functional changes occur in the nephron at the level of the glomerulus, including glomerular hyperfiltration and hyperperfusion, before the onset of any measurable clinical changes. Subsequently, thickening of the glomerular basement membrane, glomerular hypertrophy, and mesangial expansion take place. Multiple mechanisms contribute to the development and outcomes of diabetic nephropathy, such as an interaction between hyperglycemia-induced metabolic and hemodynamic changes and genetic predisposition, which sets the stage for kidney injury. Findings from various studies support an association between increased secretion of inflammatory molecules, such as cytokines, growth factors and metalloproteinases, and development of diabetic nephropathy[2,3].

Beta2-microglobulin is a low molecular weight protein normally cleared by the kidneys at a rate comparable to GFR, then reabsorbed and catabolized in the tubules and serum levels are inversely related to GFR[4]. Chemerin is a novel adipokine that regulates adipocyte development and metabolic function as well as glucose metabolism in liver and skeletal muscle

tissues. A growing body of human experimental data indicates that serum chemerin levels are elevated in patients with obesity and that they exhibit a positive correlation with various aspects of the metabolic syndrome[5,6].Therefore, the dual role of chemerin in inflammation and metabolism might provide a link between chronic inflammation and obesity, as well as obesity-related disorders such as type 2 diabetes and its microvascular complications including diabetic nephropathy. Thus, the aim of this work was to study serum chemerin and beta2-microglubulin in diabetic patients with different stages of diabetic nephropathy, in a trial to explore their correlation with kidney functions and their value in assessment of disease severity.

2.Subjects and Methods

I. Subjects:

This study was conducted at the Endocrinology Department of Ain Shams University Hospitals on 60 adult patients with type 2 diabetes mellitus and 40 apparently healthy subjects serving as a control group, all of whom willingly participated in the study.

A. Patients' Group (n=60) : This group included type 2 DM patients diagnosed according to American Diabetic Association (ADA) guidelines[7].This group included 32 males and 28 females, with a mean age of 48±6 years. They were classified according to urinary albumin excretion into the following subgroups:

1-Subgroup1a(n=16):

They included patients with normoalbuminuria, UAE<30 mg/24h). They were 8 males and 8 females whose mean age of 46±5 years.

2-Subgroup1b (n=16):

This subgroup included patients with microalbuminuria, 30<UAE<300 mg/24h.They were 9 males and 7 females with mean age of 51±5 years.

3-Subgroup1c (n=28):

This subgroup included patients with macroalbuminuria, UAE>300 mg/24h. They were 16 males and 12 females with mean age of 48±6 years.

Patients on renal replacement therapy (haemodialysis or peritoneal dialysis) and patients with causes of nephropathy rather than diabetes were excluded. In addition, type 1 diabetic patients and patients with prolifrative disorders, active inflammatory diseases including pneumonia, urinary tract infections, endocarditis, sinusitis, rheumatoid arthritis and cholangitis were all excluded from the study.

B. Control Group (n=40): This group included twenty age- and sex-matched healthy subjects serving as a control group with a mean age of 49 ± 3 years.

After an informed consent, all individuals included in this study were subjected to the following:

1-Full history taking and thorough clinical examination.

2-Laboratory investigations including:

a) Fasting and 2h –post prandial blood glucose level.
b) Urinary albumin excretion (UAE).
c) Kidney function tests including serum creatinine and blood urea nitrogen (BUN).
d) Glomerular filtration rate (GFR) by corrected creatinine clearance.
e) Serum beta 2 microglobulin(B2M) level by enzyme linked immunosorbent assay (ELISA).
f) Serum chemerin level assay by enzyme linked immunosorbent assay (ELISA).

II. Samples:

A. Blood Samples:

Five milliliters of venous blood were collected under complete aseptic precautions from each subject after fasting 6-8 h into a plain test tube for serum separation. After clotting, samples were centrifuged at 1000 xg for 15 minutes, and sera were separated into 2 aliquots, one aliquot for immediate assay of fasting glucose, creatinine and BUN. The other aliquot was stored at – 20 °C for the subsequent assay of serum B2M and chemarin. Hemolysed samples were discarded, repeated freezing and thawing was avoided. A second sample was collected for 2h-post prandial blood glucose.

B. Urine Samples:

Two milliliter (2 mL) of 24 hours freshly voided urine samples were collected. Patients were instructed to record the start and end time before starting urine collection. Microbially contaminated or turbid samples were not suitable for nephelometric measurements. So, these samples have been centrifuged at 1000 xg for 10 minutes. The 24 hours urine samples were used for immediate estimation of urinary creatinine used in corrected creatinine clearance measurement as well as for 24 hours microalbumin measurement in all subjects.

III. Methods:

A. Analytical Methods:

1. Fasting and 2h-post prandial serum glucose: were measured on Synchron CX9 autoanalyzer (Beckman Instruments Inc.; Scientific Insturments Division, Fullerton, CA 92634, 3100.
2. Urinary albumin excretion: 24 hours urinary microalbumin was determined using reagents provided from MININEPH^{TM} HUMAN MICROALBUMIN KIT (8 Calthrope Road, Edgbaston, Birmingham, UK).The determination of soluble antigen concentration by nephelometric methods involves a reaction with specific antiserum to form insoluble complexes. When light is passed through the suspension formed, a portion of the light is scattered and detected by a photodiode and the amount of light scattered is directly proportional to the specific protein

concentration in the test sample. Then concentrations are automatically calculated by reference to a calibration curve within the instrument [8].Results were calculated by the MININEPH and displayed in mg/L. The 24 hours mg microalbumin was calculated by multiplying the result in mg/L by urine volume in liters.

3. Routine kidney function tests: Routine Kidney function tests (serum creatinine and BUN) were measured on Synchron CX9 autoanalyzer (Beckman Instruments Inc.; Scientific Insturments Division, Fullerton, CA 92634, 3100, USA).
4. GFR measurement using corrected creatinine clearance: It was calculated using the following equation: $(U(mg\ /\ dL) \times V(ml\ /\ \min\ ) \times 1.73)/(S\ (mg\ /\ dL) \times A)$

   Where (V) is the volume of urine measured in (mL), urine flow rate is calculated (mL/min) and creatinine is measured in mg/dL in both urine (U) and serum (S) specimens. Then the calculated clearance is multiplied by 1.73/A, which represents the body surface area of an average-sized individual divided by the individual's body surface area in square metres (A), as determined from a nomogram that uses weight and height measurements to determine the surface area, and creatinine clearance is then reported as mL/min/1.73m^2[9].
5. Serum Beta 2- Microglobulin (B2M): It is measured using indirect solid phase enzyme immunoassay (ELISA) provided by ORG 5BM KIT (ORGENTEC Diagnostica GmbH, Carl-Zeiss-Strabe 49 55129 Mainz-Germany).This assay is a sandwich ELISA based, in which a highly purified anti human antibodies are bound to microwells. If B2M present in serum it would bind to the respective antibody. This was followed by washing of the microwells to remove unspecific components. Then horseradish peroxidase (HRP) conjugate was added. After another washing step an enzyme substrate was added to form a colour in the presence of the bound conjugate. Then an acid was added to stop the reaction forming a colored end product. The intensity of this colour was measured photometrically at 450 nm at which the amount of the colour is directly proportional to the concentration of B2M present in the original sample [10].The results were calculated in µg/mL using a calibrator curve constructed by plotting the absorbance (Y) of standards against log of known concentration (X) of standards.
6. Serum Chemerin: was measured using reagents provided from HUMAN CHEMERIN ELISA KIT (Millipore.6 Research Park Drive. St. Charles, Missouri 63304 USA).This assay is a Sandwich ELISA based, in which human chemerin molecules from samples were captured to the wells of a microtiter plate coated by a pre-tittered amount of anti-human chemerin antibody. Then the unbound materials from samples were washed away. This was followed by binding of a biotinylated anti-human chemerin antibody to the captured molecules. After another washing step, horseradish peroxidase conjugate was added. Then free enzyme conjugates washed away, and quantification of immobilized antibody-enzyme conjugates by monitoring horseradish peroxidase activities in the presence of the substrate 3,3',5,5'-tetramethylbenzidine. The enzyme activity is measured spectrophotometrically by the increased absorbance at 450 nm, after acidification of formed products. Since the increase in absorbance is directly proportional to the amount of captured human chemerin in the unknown sample, the latter can be derived by interpolation from a reference curve generated in the same assay with reference standards of known concentrations of human chemerin expressed in ng/mL [11].

B) Statistical Methods:

IBM SPSS statistics (V. 21.0, IBM Corp., USA, 2012) was used for data analysis. Parametric data were expressed as mean and standard deviation ($\bar{X}$ ± SD) while non-parametric data were expressed as median and interquartile range (IQR). Comparative statistics was done by Wilcoxon's rank sum and Kruskal Wallis test in case of non-parametric data whereas Student t test ,one –way ANOVA and multiple comparison test for parametric data. Correlation analysis was performed by Spearman's rank correlation (r_S). *P* values<0.05 were considered significant, whereas values<0.01 were considered highly significant.

3. Results

Results are presented in tables (1-4) and figures (1-3).

Comparing diabetic patients with controls, type2 diabetics had significant higher 2h-post prandial glucose (180.86±21.11mg/dL) vs.(100.39±17.70 mg/dL; p<0.01) in control group , higher UAE [206.00 (59.00-510.00 mg/24h)] vs.[22.00 (16.50-25.00 mg/24h)],higher Bun [18 (14-46 mg/dL)] vs.[12 (10-14mg/dL)], higher serum creatinine [1.10(0.80-1.95mg/dL)] vs [0.7 (0.60-0.90mg/dL)], higher serum chemerin [200.00(65.00-300.00ng/mL)]vs. [35.00 (25.00-42.50 ng/mL)], higher B2M [5.00(2.75-8.00µg/mL)] vs.[2.00 (1.50-2.35µg/mL)] and significantly lower corrected creatinine clearance [89(24-100 mL/min/1.73m^2)] vs. [113 (105-120mL/min/1.73m^2)] in control group (p<0.01; respectively; **Table 1**).

On comparing patients' subgroups versus controls, a highly significant increase was found in serum levels of fasting glucose, 2h-pp glucose, creatinine, BUN,

chemerin and B2M in macroalbuminuric patients (subgroup 1c) compared to controls (p<0.01; respectively). In addition, a highly significant elevation was detected in serum levels of chemerin as well as B2M in patients with microalbuminuria (subgroup 1b) versus control group (z= 3.78,3.59; p<0.01; respectively). However, no significant difference was observed in serum chemerin nor serum B2M between normoalbuminuric patients(subgroup 1a) compared to control (p>0.05) (**Table 2**).

As regards corrected creatinine clearance, a highly significant decrease was observed in patients with macroalbuminuria (subgroup 1c) versus control group (p<0.01). However, no significant difference was detected in other subgroups (sub groups 1a, 1b) versus control group (p>0.05, respectively; **Table 2**).

Table 3 and **Figure 1** show comparative analysis between all studied parameters among patients' subgroups. A highly significant difference was observed between all patients' subgroups for all studied parameters (p<0.01) with highest value of Kruskall Wallis test for serum chemerin level (H=22.15) in differentiating between the three subgroups. Moreover, a highly significant elevation was observed in serum chemerin and serum B2M in patients with microalbumiuria compared to normoalbuminuria (z=3.249, 3.133; p<0.01; respectively).

Furthermore, a significant elevation was observed in serum levels of fasting glucose, 2h-pp glucose, creatinine, BUN, chemerin and B2M in patients with macroalbuminuria compared to patients with normoalbuminuria and patients with microalbuminuria (p<0.01; respectively).On the other hand, corrected creatinine clearance showed a highly significant decrease in patients with macroalbuminuria compared to patients with normoalbuminuria as well as patients with microalbuminuria (z=-3.658,-3.823; p<0.01; respectively) (**Table 3**).

Correlation study in patients' group revealed a highly significant positive correlation between serum chemerin and serum B2M (r_s=0.818, p<0.01). In addition, Serum levels of chemerin and B2M showed a significant positive correlation with serum 2h-pp glucose, creatinine, BUN and UAE (p<0.01; respectively). On the other hand, values of corrected creatinine clearance showed a highly significant negative correlation with serum levels of chemerin and B2M (r_s=-0.769.-0.659; p<0.01; respectively) (**Table 4**).

As regards correlation with UAE in patients' subgroups, serum chemerin showed a significant positive correlation with UAE in patients with normoalbuminuria (r_s=0.826, p<0.05; **Figure 2**) and a highly significant positive correlation with UAE in patients with microalbuminuria (**Figure 3**) and patients with macroalbuminuria (r_s=0.934, 0.919; p<0.01; respectively). However, B2M showed a significant positive correlation with UAE in patients with macroalbuminuria only (r_s=0.506, p<0.05).

Table (1):Descriptive and Comparative Statistics of All Studied Parameters in Patient Group Compared to Control Group

Parameter	Type 2 DM Patients (n = 60)	Control Group (n = 40)	t*/z	p-value
	$\bar{x}$ ±SD* / Median (IQR)	$\bar{x}$ ±SD* / Median (IQR)		
Age (years)	48.52±6.14*	49.46±3.89*	-0.602*	>0.05
FBS (mg/dL)	98.72±20.97*	86.00±9.37*	1.964*	<0.05
2h-PP (mg/dL)	180.86±21.11*	100.39±17.70*	2.305*	<0.05
UAE (mg/24 h)	206.00(59.00-510.00)	22.00 (16.50-25.00)	4.602	<0.01
BUN (mg/dL)	18.00(14.00-46.00)	12.00 (10.00-14.00)	3.645	<0.01
Serum Creatinine (mg/dL)	1.10(0.80-1.95)	0.70 (0.60-0.90)	2.950	<0.01
Corrected creatinine clearance (ml/min/1.73m^2)	89(24-100)	113 (105-120)	-3.774	<0.01
Serum Chemerin (ng/mL)	200.00(65.00-300.00)	35.00 (25.00-42.50)	3.941	<0.01
Serum B2-microglobulin (µg/mL)	5.00(2.75-8.00)	2.00 (1.50-2.35)	3.528	<0.01

$\bar{x}$ ±SD* = Mean±SD ,Median. (IQR) = Median (Interquartile range), Z = Wilcoxon Rank sum test, t* = Student t-test , UAE = Urinary albumin excretion, p>0.05:Nosignificant difference, p<0.05 :Significant difference, p<0.01: Highly significant difference

Table (2):Descriptive and Comparative Analysis of All Studied Parameters in Patient Subgroups Compared to Control Group

Parameter	Normo-albuminuria (subgroup Ia) (n = 16)	Micro-albuminuria (subgroup Ib) (n = 16)	Macro-albuminuria (subgroup Ic) (n = 28)	Control Group (n=40)	Normo vs. control		Micro vs. control		Macro vs. control	
	$\bar{x}$ ±SD / Median (IQR)	$\bar{x}$ ±SD / Median (IQR)	$\bar{x}$ ±SD / Median (IQR)	$\bar{x}$ ±SD / Median (IQR)	t/z	*p*	t/z	P	t/z	*p*
FBG (mg/dL)*	88.29±9.95	98.8±6.4	107.1±23.1	86.00±9.37	0.50	>0.05	2.10	<0.05	3.14	<0.01
2h-PP (mg/dL)*	172.71±14.16	165.9±13.5	193.5±20.6	100.39±17.70	5.36	<0.01	5.02	<0.01	5.52	<0.01
BUN (mg/dL)	15.0 (13.0-18.0)	12.0 (11.3-17.3)	46.0 (35.0-53.0)	12 (10-14)	2.36	<0.05	0.95	>0.05	4.42	<0.01
Serum creatinine (mg/dL)	0.8 (0.5-0.9)	0.9 (0.7-1.0)	2.0 (1.5-3.2)	0.70 (0.60-0.90)	0.20	>0.05	1.43	>0.05	4.42	<0.01
Corrected creatinine clearance (mL/min/1.73m^2)	100 (95-112)	99 (91-113)	24 (16-44)	113 (105-120)	-1.87	>0.05	-1.74	>0.05	-4.42	<0.01
Serum chemerin (ng/mL)	35.0 (30.0-40.0)	155.0 (95.0-197.5)	300.0 (237.5-393.8)	35 (25-42.5)	0.12	>0.05	3.78	<0.01	4.43	<0.01
Serum B2-microglobulin (µg/mL)	1.5 (1.0-2.2)	4.5 (3.5-6.0)	7.8 (5.4-10.0)	2.00 (1.50-2.35)	0.88	>0.05	3.59	<0.01	4.42	<0.01

$\bar{x}$ ±SD = Mean±SD used for parametric data*.Median. (IQR) = Median (Interquartile range).
Z = Wilcoxon Rank sum test, t = Student t-test for parametric data*, UAE = Urinary albumin excretion
p>0.05:Nosignificant difference, *p*<0.05 :Significant difference, *p*<0.01: Highly significant difference

Table (3):Comparative Analysis between All Studied Parameters among Patient Subgroups

Parameter	F*/H	*P*	Normo vs. Micro		Normo vs. Macro		Macro vs.Micro	
			$\bar{x}$ diff.**/z	*P*	$\bar{x}$ diff.**/ z	*P*	$\bar{x}$ diff.**/z	*P*
FBG(mg/dL)	7.402*	<0.01	-9.53*	>0.05	-18.78*	<0.05	28.32*	<0.01
2h-PP (mg/dL)	7.331*	<0.01	-6.83*	>0.05	-20.78*	<0.05	27.62*	<0.01
BUN (mg/dL)	21.63	<0.01	-1.36	>0.05	-3.66	<0.01	3.83	<0.01
Serum Creatinine (mg/dL)	21.55	<0.01	-1.293	>0.05	-3.66	<0.01	3.826	<0.01
Corrected creatinine clearance(ml/min/1.73m^2)	21.08	<0.01	0.059	>0.05	3.658	<0.01	-3.823	<0.01
Serum Chemerin (ng/mL)	22.15	<0.01	-3.249	<0.01	-3.671	<0.01	3.397	<0.01
Serum B2 – microglobulin(µg/mL)	18.82	<0.01	-3.133	<0.01	-3.665	<0.01	2.503	<0.05

$\bar{x}$ ±SD = Mean±SD for parametric data, Median. (IQR) = Median (Interquartile range).
UAE = Urinary albumin execretion
H = Kruskall Wallis test F* = ANOVA test for parametric data
Z = Wilcoxon Rank Sum test. $\bar{x}$ Diff.** = Mean difference by multiple comparison test
p>0.05: No significant difference, p<0.05 Significant difference, p<0.01 Highly significant difference

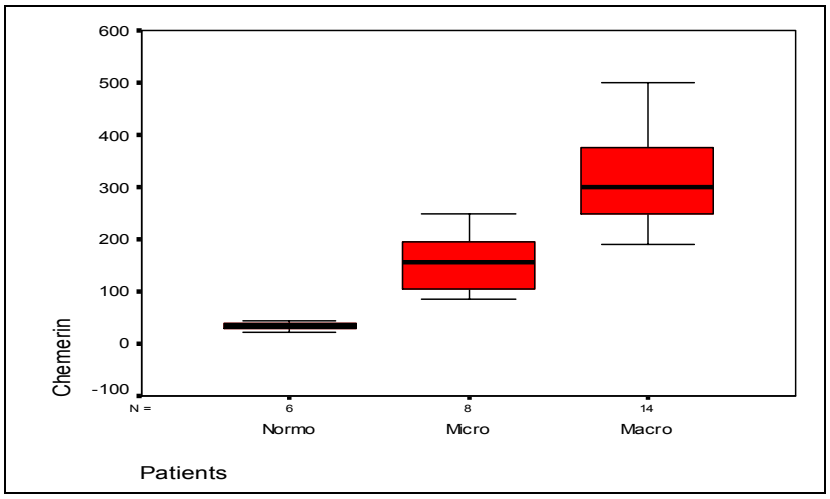

Figure (1):Differences between Patient Subgroups Concerning Serum Chemerin Level in ng/mL

Table 4:Correlation Study between Serum Chemerin,Beta2-Microglubulin and all Studied Parameters in Patient Group

Parameters	Chemerin		Beta 2-Microglobulin	
	r_s	*p*-value	r_s	*p*-value
Age (years)	0.203	>0.05	0.337	>0.05
FBS (mg/dL)	0.405	<0.05	0.272	>0.05
2h-PP (mg/dL)	0.489	<0.01	0.422	<0.05
UAE (mg/24 h)	0.975	<0.01	0.829	<0.01
BUN (mg/dL)	0.643	<0.01	0.535	<0.01
Cr (mg/dL)	0.758	<0.01	0.622	<0.01
Corrected creatinine clearance (ml/min/1.73m^2)	-0.769	<0.01	-0.659	<0.01
Serum Chemerin (ng/mL)	-	-	0.818	<0.01

r_s = Ranked Spearman Correlation Test, p> 0.05 :Non significant, p< 0.05:Significant ,p<0.01 :Highly significant correlation

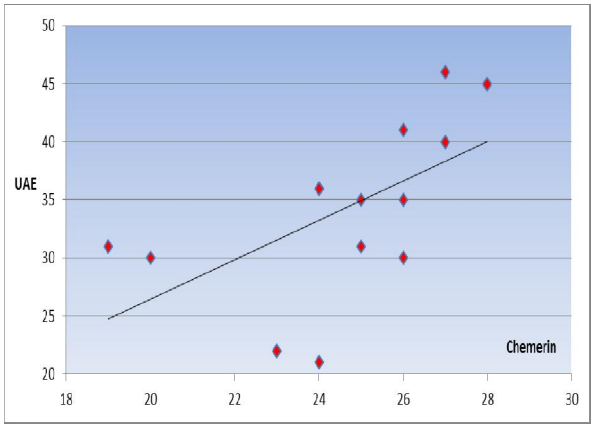

Figure (2): Correlation between Serum Chemerin and UAE in Normoalbuminuric Subgroup

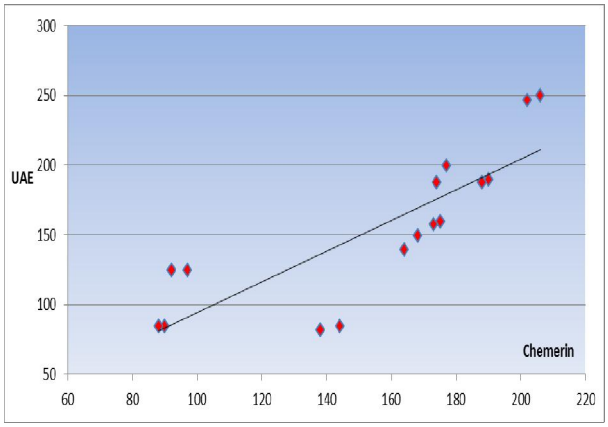

Figure (3): Correlation between Serum Chemerin and UAE in Microalbuminuric Subgroup

4. Discussion

Diabetic nephropathy, a serious microvascular complication is recorded in approximately one third of patients with diabetes. It is considered the most common cause of end-stage renal disease[12,13]. Diabetic nephropathy affects all the kidney cellular elements, that is, glomerular endothelia, mesangial cells, podocytes, and tubular epithelia. It is characterized by excessive accumulation of extracellular matrix with thickening of glomerular and tubular basement membranes and increased amount of mesangial matrix, which ultimately progresses to glomerulosclerosis and tubulointerstitial fibrosis[14].

Circulating chemerin, a novel adipokine linked to obesity, glucose tolerance and hyperlipidemia, was recently reported to be increased in chronic kidney disease patients. Therefore, serum chemerin concentration might be altered in patients with diabetic nephropathy[15]. Beta2-microglobulin belongs to low molecular weight proteins (11.8 KD). It is produced by all nucleated cells but lymphocytes are the main site of its synthesis. Malfunction of proximal convoluted tubules as in diabetic nephropathy will be accompanied by decrease tubular reabsorption of β2-M and increase its urinary excretion[16].

In the view of the previous studies, we aimed to study serum chemerin levels and beta2-microglobulin to determine their association with kidney functions in patients with type 2 diabetes mellitus. As microalbuminuria is the earliest clinically detectable stage of diabetic kidney disease at which appropriate interventions can retard or reverse the progress of the disease, we classified the diabetic patients in relation to their urinary albumin excretion.

The present study demonstrated that diabetic patients had significant higher serum creatinine and BUN when compared to control group. These results agreed with previous studies that attributed such findings to the presence of hyperglycemia, glomeruler hypertension, advanced glycation end products (AGE), activation of polyol pathway, and infiltration of kidney glomeruli by inflammatory cells like monocytes and macrophage. They stated that the previous factors result in glomerular injury and tubulo-interstitial damage that decrease the functional capability of kidney to excrete waste products[17,18].

As regard serum chemerin, in our study, diabetic patients had significant higher serum chemerin compared to normal controls. In addition, a significant positive correlation has been found between serum chemerin levels and fasting and post prandial blood glucose, in similarity to **Albert** study[19] who reported that patients with type 2 diabetes had significantly higher levels of chemerin than non diabetic patients. ***Sell and his colleagues*** attributed this increase to the fact that chemerin induces insulin resistance in peripheral tissues as skeletal muscle and inhibits glucose uptake[20]. However, another explanation postulated that, in adipocytes, chemerin has the opposite effect, where it increases insulin-stimulated glucose uptake, and so, it stimulates insulin sensitivity. Hence, the increase in the levels of circulating chemerin is a compensatory mechanism in patients with insulin resistance[21]. Conversely, ***Yamamoto et al.*** concluded that serum chemerin levels were lower in diabetic patients compared to non-diabetics, in a study of diabetic patients on dialysis, and found that elevated chemerin was associated with a survival advantage[22]. These observations necessitate more work on different stages of diabetic nephropathy with follow up and mortality risk assessment.

Interestingly, elevated serum chemerin levels demonstrated no gender differences in the diabetic patients in this study, making it superior to serum creatinine. This observation was in agreement with the findings of previous studies that also failed to find a significant difference in serum chemerin levels between the female and male subjects[23,24]. By contrast, ***Bozaoglu et al.*** reported higher chemerin levels in females compared with males[25]. This inconsistency may have resulted from the environmental and ethnic differences between the populations or differences in sample collection and storage.

In the present study, diabetic patients had significant higher serum creatinine, BUN and UAE compared to control group and that diabetic patients with macroalbuminuria had significantly higher fasting glucose, 2h-pp ,serum creatinine, serum chemerin and beta 2- microglobulin in addition to significantly lower corrected creatinine clearance than patients with microalbuminuria and normoalbuminurea. Moreover, a highly significant positive correlation was observed between serum chemerin and UAE , BUN and creatinine in diabetic patient group. Furthermore, serum chemerin showed a highly significant negative correlation with corrected creatinine clearance. These findings were in agreement with and explained by several studies stated that in advanced stages of diabetic nephropathy, there is more glomerular enlargement as a compensatory mechanism to overcome glomerulopathy, leading to more loss of kidney function and more albumin excretion through increased permeability to albumin. This was associated with impaired clearance of chemerin that may lead to the accumulation of chemerin in the blood [26,27,28,29], confirming the possible use of serum chemerin as a diagnostic marker for diabetic nephropathy.

Obviously, the present study revealed that serum chemerin was the most significant differentiating marker among the studied parameters in

discrimination between patient subgroups (H=22.15, $p<0.01$). Moreover, the present study revealed a highly significant positive correlation between serum chemerin and UAE in diabetic patient group as well as within the subgroups (normoalbuminuria, microalbuminuria, macroalbuminuria). This finding suggested the use of serum chemerin as a predictor for development of diabetic nephropathy. However, this finding was against previous studies that failed to find a significant difference in serum chemerin levels between normal controls and patients with microalbuminuria[23,25], given that quite probably a proportion of their type 2 diabetic study subjects may be controlled well by anti-diabetic drugs, that was not the case in the present study.

Moreover, a highly significant positive correlation has been found between serum chemerin and serum beta 2- microglobulin. This finding in the present study was the first work that linked a tubular function test (beta 2- microglobulin) with adipokine marker (chemerin) which represent the relationship between increased uraemic fat mass and inflammation as well as tubular dysfunction in patient with diabetic nephropathy.

Concerning beta 2- microglobulin, the present study showed a highly significant elevation in beta 2-microglobulin in diabetic patient group and subgroups (microalbuminuria and macroalbuminuria) compared to control group. These findings were in accordance with previous studies revealing its association with renal dysfunction[4,30]. In addition, a highly significant correlation was found between serum beta 2-microglobulin and UAE in patient group as well as patient with macroalbuminuria, in agreement with previous studies[16,30]. Beta 2- microglobulin is a low molecular weight protein that is released at constant rate and filtered by the glomerulus, absorbed and catabolised by proximal tubules. Therefore, it is theoretically considered a suitable biomarker of renal dysfunction. In fact, tubular involvement may precede glomerular involvement because several of these tubular proteins and enzymes are detectable even before the appearance of microalbuminuria and rise in serum creatinine[30].

Furthermore, a significant positive correlation was found between serum beta 2- microglobulin and serum creatinine and BUN in addition to a significant negative correlation with corrected creatinine clearance. Several studies were in accordance with these findings[4,16,30]. Changes in glomerular filtration rate provide a valuable indicator of the progression of diabetic nephropathy and beta2-microglobulin in diabetics may be an early indicator of incipient diabetic nephropathy. They added that serum beta 2-microglobulin was more sensitive and accurate for assessment of renal functions as compared to serum creatinine, as serum creatinine does not reflect any renal damage in early stages of diabetes owing to its secretion by renal tubules. Therefore, beta2-microglobulin is superior to that of serum creatinine in distinguishing between mild and moderately reduced GFR[4,16,30].

In our study, both chemerin and beta2-microglobulin were elevated stepwise from normoalbuminuric stage to microalbuminuria and macroalbuminuria with a statistically highly significant increase in their serum levels as diabetic nephropathy progress to overt nephropathy, indicating the possibility of introducing both markers in a panel to assess disease severity especially if correlated with histopathological changes in early stages of diabetic nephropathy development.

In conclusion, increased serum chemerin and beta2-microglubulin may be early indicators of incipient diabetic nephropathy in type 2 diabetic patients as both were significantly correlated with kidney functions including urinary albumin excretion and glomerular filtration rate. Moreover, combined use of both markers could be used in assessment of disease severity.

References

1. Arora MK, Singh UK. Molecular mechanisms in the pathogenesis of diabetic nephropathy: an update. Vascul Pharmacol 2013;58(4):259-71.
2. Dronavalli S, Duka I, Bakris GL. The pathogenesis of diabetic nephropathy. Nat Clin Pract Endocrinol Metab.2008; 4(8): 444-452.
3. Susztak k and Bottinger EP. Diabetic nephropathy: a frontier for personalized medicine. J Am Soc Nephrol 2011; 17:361–367.
4. Wibell L. The serum level and urinary excretion of beta 2 microglobulin in health and renal disease. Pathol Biol 2010 ;26:295-301
5. Lehrke M, Becker A, Greif M, Stark R, Laubender RP, et al. Chemerin is associated with markers of inflammation and components of the metabolic syndrome but does not predict coronary atherosclerosis. Eur J Endocrinol.2009; 161(2): 339-344.
6. Ernst MC, Issa M, Goralski KB and Sinal CJ. Chemerin exacerbates glucose intolerance in mouse models of obesity and diabetes. Endocrinology 2010; 151(5): 1998-2007.
7. American Diabetes Association:Diagnosis of diabetes mellitis.Diabetes Care 2012; 1(27): 57-59.
8. Florvall G, Basu S, Helmersson J, Larsson A. Microalbuminuria measured by three different methods, blood pressure and cardiovascular risk factors in elderly Swedish males. Anal Chem Insights. 2008; 3: 69–74.

9. Bazari H. Approach to the patient with renal disease. In: Cecil Medicine. (23rd ed). H Bazari (Eds).Philadelphia, Pa: Saunders Elsevier. 2010, pp:456- 478.
10. Aminian O, Eftekhari S, Mazaheri M, Sharifian SA, Sadeghniiat-Haghighi K. Urinary β2 Microglobulin in Workers Exposed to Arc Welding Fumes. Acta Medica Iranica, 2011; 49(11): 748-752.
11. Goralski KB, McCarthy TC, Hanniman EA, Zabel BA, Butcher EC, et al. Chemerin, a novel adipokine that regulates adipogenesis and adipocyte metabolism. J Biol Chem 2007; 282: 28175-28188.
12. Shlipak M. Diabetic nephropathy. Am J Kidney Dis 2009; 54:619–637.
13. Susztak k and Bottinger EP. Diabetic nephropathy: a frontier for personalized medicine. J Am Soc Nephrol 2011; 17:361–367.
14. Cohen-Bucay A, Viswanathan G. Urinary markers of glomerular injury in diabetic nephropathy. International Journal of Nephrology2012; http://dx.doi.org/10.1155/2012/146987
15. Pfau D, Stepan H, Kratzsch J, Verlohren M, Verlohren HJ, et al. Circulating levels of the adipokine chemerin in gestational diabetes Mellitus. Horm Res Paediatr. 2010; 79: 101-108.
16. Shahjahan, Javaid H ,Jawaid S. Beta 2 microglobulin and cystatin c in type 2 diabetes. Gomal Journal of medical sciences 2012;9(2):87-95.
17. Priyanka T, Zenith K, Satyavani K, Mary B , Vijay V. Clinical significance of urinary Monocyte Chemo-attractant Protein-1 (uMCP-1) in Indian type 2 diabetic patients at different stages of diabetic nephropathy. International Journal of Diabetes Mellitus 2010; 2: 15–19.
18. Aronson D, Mittleman MA , Burger AJ. Elevated blood urea nitrogen level as a predictor of mortality in patients admitted for decompensated heart failure. Am J Med 2010;116:466–73.
19. Albert H. Low omentin-1, high chemerin levels linked to Type 2 diabetes. Diabet Med 2011; Advance online publication.
20. Sell H, Laurencikiene J, Taube A, Eckardt K, Cramer A, et al. Chemerin is a novel adipocyte-derived factor inducing insulin resistance in primary human skeletal muscle cells. Diabetes 2009; 58(12): 2731-2740.
21. Takahashi M, Takahashi Y, Takahashi K, Zolotaryov FN, Hong KS, *et al*. Chemerin enhances insulin signaling and potentiates insulin-stimulated glucose uptake in adipocytes. FEBS Lett.2008; 582(5): 573-578.
22. YamamotoT, QureshiAR, Anderstam B, Heimbürger O, BárányP *et al.* Clinical importance of an elevated circulating chemerin level in incident dialysis patients. Nephrology Dialysis Transplantation 2010; 25(12):4017-4023.
23. Pfau D, Bachmann A, Lössner U, Kratzsch J, Blüher M, Stumvoll M, et al. Serum levels of the adipokine chemerin in relation to renal function. Diabetes Care. 2010;33:171–173
24. Stejskal D, Karpisek M, Hanulova Z and Svestak M. Chemerin is an independent marker of the metabolic syndrome in a Caucasian population – a pilot study. Biomed Pap 2008; 152(2):217-221.
25. Bozaoglu K, Segal D, Shields KA, Cummings N, Curran JE, et al. Chemerin is associated with metabolic syndrome phenotypes in a Mexican-American population. J Clin Endocrinol Metab 2009; 94:3085–8.
26. Murata M, Saito T, Otani T, Sasaki M, Ikoma A, *et al.* An increase in serum retinol-binding protein 4 in the type 2 diabetic subjects with nephropathy. Endocr J 2009;56:287–94.
27. Pajica P and Zelijco M. Microalbuminuria and Diabetes melliteus. Diabetologia cortica 2009; 31-44.
28. Hu W and Fing P. Elevated serum chemerin concentrations are associated with renal dysfunction in type 2 diabetic patients. Diabetes research and clinical practice 2010;91(2):159-163.
29. Dorte P, Anette B, Matthias B, Micheal S and Jurgen K. Serum levels of the adipokine chemerin in relation to renal function. Diabetes care2012 ;33(1):171-173.
30. Latha T, Jaganmohan P and Subramanyam P. Evaluation of diabetic nephropathy using selected biochemical markers in Settiyar (Vysya) Community of Nelloreand Parkasam Districts of Andhra Pradesh, India. Advances in Biological Research 2012; 6 (2): 81-86.

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