

Role of serum IL-18 in type 2 diabetic patients with and without microalbuminuria

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Abstract

Background: Microalbuminuria is a well-known indicator of poor renal outcomes in patients with type 2 diabetes that leads to diabetic nephropathy (DN), but there are many proinflammatory markers that are elevated during injury to the nephrons which lead to chronic kidney disease, one of those is interleukin - 18 (IL-18).

Objective: To confirm the role of serum IL-18 as a novel predictor before DN onset.

Patients and Methods: The microalbuminuria levels were measured by immunoturbidimetric method, serum IL-18 levels were measured by ELISA sandwich method, both serum and urine creatinine levels were measured by colorimetric method and hemoglobin A1c (HbA1c) levels were measured by ion exchange - high performance liquid chromatography (HPLC) in 90 individuals, 20 individuals apparently healthy (group I), 40 diabetic patients without microalbuminuria (urinary albumin to creatinine ratio (UACR) < 30 mg/g) (group II) and 30 diabetic with microalbuminuria (UACR 30 -300) (group III).

Results: The mean of serum IL-18 in group I, II and III was 189.30 pg/ml \pm 3.269 S.E., 220.83 pg/ml \pm 0.900 S.E. and 246.67 pg/ml \pm 22.341 S.E. respectively. The difference between group I and II, group I and III, and group II and III was significant (P=0.000 for all). The mean of UACR in group I, II and III was 4.25 mg/g \pm 2.359 S.E., 4.20 mg/g \pm 1.518 S.E. and 34.17 mg/g \pm 0.629 S.E. respectively. The difference between group I and II was not significant (P=1.000) but between group I and III, and group II and III were significant (P=0.000 for both). The mean of serum creatinine in group I, II and III was 0.885 mg/dl \pm 0.0302 S.E., 0.883 mg/dl \pm 0.0240 S.E. and 0.933 mg/dl \pm 0.0237 S.E. respectively. The difference between group I and II, group I and III, and group II and III was not significant (P=0.998, 0.331 and 0.145 respectively). The mean of hemoglobin A1c (HbA1c) in group I, II and III was 5.9 % \pm 0.0211 S.E., 7.7 % \pm 0.0445 S.E. and 8.135 % \pm 0.0519 S.E. respectively. The difference between group I and II, group I and III, and group II and III was significant (P=0.000 for all). The correlation between urinary albumin to creatinine ratio (UACR) and IL-18 in group III was significant (r=0.983 with P=0.000). The correlation between hemoglobin A1c and IL-18 was significant (r=0.641 with P=0.000) in group II. The correlation between serum creatinine and IL-18 in group III was not significant (r=0.041 with P=0.830).

Conclusion: while the increased IL-18 levels were positively correlated with both HbA1c and UACR that leads to the progression of the diabetic nephropathy and it can be one of the cytokines which opens the possibility of its application in clinical treatment in the future.

Keywords: IL-18, Microalbuminuria, Diabetic Nephropathy.

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INTRODUCTION

IL-18 is a potent inflammatory cytokine, also known as interferon-gamma inducing factor (IGIF) produced by

the cells of the monocytic lineage and dendritic cells that promotes differentiation of TH1 subset of helper T cells, stimulates interferon gamma (IFN-gamma) production and enhancement of natural killer cell activity, its

molecular weight is 18.2 kDa [1]. IL-18 has a structure determinable similar to IL-1 [2 & 3] and functional properties with IL-12 [3]. A study was achieved in 2002 demonstrated that IL-18 has a significant pathological role to induce acute renal failure (ARF) [4]. In a study done by Moriwaki [5] stated that serum levels of IL-18 were increased in diabetic patients with proteinuria compared with those without proteinuria. When urinary albumin to creatinine ratio (UACR) is greater than 30 mg/g it will be a marker for CKD, UACR is unaffected by variation in urine concentration [6]. Hemoglobin A1c (HbA1c) is presently the most commonly used marker for the determination of the glycemic status in diabetics [7], as well as it can be applied as indicator to monitor the microvascular diabetic complications (retinopathy, nephropathy, and neuropathy) [8]. Our objective was to confirm the role of serum IL-18 as a novel predictor before diabetic nephropathy (DN) onset.

PATIENTS AND METHODS

This study involved 90 individuals, 20 individuals apparently healthy (group I), 40 diabetic patients without microalbuminuria (urinary albumin to creatinine ratio (UACR) < 30 mg/g) (group II) and finally 30 diabetic with microalbuminuria (UACR 30-300) (group III). All these patients attended the National Diabetic Center in Baghdad and were measured for fasting blood sugar and body mass index and they did not have allergy, non smoking and with normal blood pressure. Mean duration of diabetes in group II was 4.27 years \pm 0.521 S. E. . Mean duration of diabetes in group III was 8.93 years \pm 0.442 S.E.. The group I included 10 female and 10 male, with the mean age of 53.8 years \pm 1.988 S.E., group II included 18 female and 22 male, with the mean age of 55 years \pm 1.493 S.E., group III included 15 female and 15 male, with the mean age of 58.6 years \pm 1.395 S.E.. The average hemoglobin A1c (HbA1c) in group I was 5.9 % \pm 0.0211 S.E., in group II was 7.7 % \pm 0.0445 S.E. and in group III was 8.135 % \pm 0.0519 S.E.. All serum samples obtained from the three groups were examined by using the routine methods for (C-reactive protein (CRP), erythrocyte sedimentation rate (ESR) and lipid profile) requested by the center to exclude all the cases with abnormal findings. All subjects of group I, II and III were excluded from any chronic disease (hypertension, heart attack, stroke, rheumatoid arthritis, etc.)

General Urine Examination:-

This test was done by following the conventional common method. The aim of doing this simple test was to exclude all cases with abnormal urine findings (erythrocytes, leukocytes and active sediments).

Microalbuminuria:-

Semi-quantitative method:-

Microalbuminuria was tested by dip strips that is reacted with Sulfonephthalein 2.2% as a reagent to yield sky like color ranged from mild, moderate to severe cases which is considered as semi quantitative measurement 30-80 mg/L, 81-150 mg/L and >150 mg/L respectively. The procedure is used only when the sample was well mixed uncentrifuged urine and which should not be older than two hours. First morning urine is recommended. The samples were protected from light. The test strip was immersed in the urine (approximately 2 seconds), so that all reagent areas are covered. Excess urine was removed from the strip by wiping the edge of the strip on the urine container or on absorbent paper. The reagent areas were compared to the strip with the corresponding charts of color fields on the container 60 seconds after immersion. Coloration is not relevant when it was on the rim of the test pad or after 2 minutes of immersion and should not be used for interpretation [9].

Quantitative method:-

For quantitative measurement, the microalbuminuria was assayed with Cciel apparatus by immunoturbidimetric method as follows:-

Antiserum, buffer & microalbumin standard were brought to room temperature prior to measurement. The instrument was nullified with distilled water. Nine hundred 900 μ l of buffer and 60 μ l of microalbumin standard or sample were pipetted into cuvette. Cuvette was mixed and inserted into the photometer, the absorbance A1 was read at 340 nm, 150 μ l of antiserum was added, mixed and incubated for 5 minutes at room temperature, the absorbance A2 was read at 340 nm. The difference between A2 and A1 was calculated which represented by ($\Delta A = A2 - A1$) [10]. Albumin concentration in the sample was calculated by interpolation of its absorbance from the calibration curve [11].

Urine Creatinine:-

Serum creatinine was measured by a colorimetric method with Cciel apparatus as follows: the urine creatinine should be diluted by distilled water 49 times before be applied; one hundred microliter of diluted sample or standard (STD) was mixed with 1000 μ l of working reagent that was constituted from mixture in equal amount between picric acid (26 mmol/l) and diluted NaOH(1.6 mol/l), diluted NaOH was prepared by mixing one volume of NaOH and 4 volume of distilled water. After 30 seconds the absorbance A1 was read. The absorbance A2 was exactly read after 2 minutes. Creatinine forms in alkaline solution an

orange-red colored complex with picric acid. The absorbance of this complex is proportional to the creatinine concentration in the sample [12]. The concentration was measured by the following equation:-

$$C = 2 \times \frac{\Delta A \text{ Sample}}{\Delta A [\text{STD}]} \quad [\text{mg/dl}]$$

C:Creatinine concentration

ΔA:A2-A1 for both sample and standard

Urinary Albumin: Creatinine (A: C) Ratio:-

This ratio is considered as one of the best accepted means for assessing urinary albumin excretion and is recommended by the National Kidney Disease Education Program [6], its advantages are to compensate for variations in urine concentration in spot-check samples (not dependent on hydration level) and is most reproducible [13]. The ratio was measured as follows:-

$$\text{UACR} = \frac{\text{Urine albumin (mg/dl)}}{\text{Urine creatinine (g/dl)}} = \text{UACR in mg/g}$$

Serum IL-18, ELISA method:-

This test was done according to the manufacturer’s leaflet (RayBiotech) [14]. In summary, the principle is as follows:-

This assay applied to the quantitative sandwich enzyme immunoassay technique in which an antibody specific for human IL-18 coated on a 96-well plate, standards or samples were pipetted into the wells and IL-18 present in a sample was bound to the wells by the immobilized antibody. The wells were rinsed and biotinylated anti-human IL-18 antibody was added. After washing away untied biotinylated antibody, horseradish peroxidase (HRP)-conjugated streptavidin was pipetted to the wells. The wells were again rinsed, a tetramethyl benezidine (TMB) substrate solution was added to the wells and color developed in proportion to the amount of IL-18 bound. The Stop Solution changed

the color from blue to yellow and the intensity of the color was measured at 450 nm.

Hemoglobin A1c estimation:-

HbA1c percentage was estimated by application of the manufacturer’s instruction [15] based on ion exchange high performance liquid chromatography (HPLC).

RESULT

Table 1 showed that serum IL-18 level among groups I, II and III were as follows: 189.30 pg/ml ± 3.269, 220.83 pg/ml ± 0.900 and 246.67 pg/ml ± 22.341 respectively. The difference between group I and II, group I and III and group II and III was significant (P=0.000, 0.000 and 0.000 respectively).

Table 1: Interleukin 18 (pg/ml) in diabetic with and without microalbuminuria groups compared to control group.

Parameter	Groups	N	Mean	Std. Error	P-value (I & II)	P-value (I & III)	P-value (II & III)
Interleukin 18 [pg/ml]	group I	20	189.30	3.269	*0.000	*0.000	*0.000
	group II	40	220.83	900			
	group III	30	246.67	22.341			

*P-value > 0.5 is not significant

The results of UACR, serum creatinine and hemoglobin A1c (HbA1c) levels among groups I, II and III shown in table 2. The UACR mean was as follow: 4.25 mg/g ± 2.359, 4.20 mg/g ± 1.518 and 34.17 ± 1.629 respectively. The difference in UACR between group I and II was not significant (P= 1.000). The difference between group I and III and group II and III was significant (P= 0.000 for both). The mean of serum creatinine in group I, II and III was 0.885 mg/dl ± 0.0302 S.E., 0.883 mg/dl ± 0.0240 S.E. and 0.933 mg/dl ± 0.0237 S.E. respectively. The difference between group I and II, group I and III, and group II and III was not significant (P=0.998, 0.331 and 0.145 respectively).

Table 2. Urinary albumin to creatinine ratio (UACR) (mg/g), S.Cr. (mg/dl) and HbA1c (%) in controls (group I) compared to diabetics with UACR <30 (group II) and diabetics UACR ≥ 30 (group III).

Groups	N	UACR Mean	S.E.	P-value	S. Cr. Mean	S.E.	P-value	HbA1c Mean	S.E.	P-value
Group I	20	4.25	2.359	P (I & II)=1.000	0.885	0.0302	P (I & II)=0.998	5.9	0.0211	P (I & II)=0.000
Group II	40	4.20	1.518	P (I & III)=0.000	0.883	0.0240	P (I & III)=0.331	7.7	0.0445	P (I & III)=0.000
Group III	30	34.17	0.629	P (II & III)=0.000	0.933	0.0237	P (II & III)= 0.145	8.13	0.0519	P (II & III)= 0.000

The HbA1c mean was as follow: 5.9 % ± 0.0211 S.E., 7.7 % ± 0.0445 S.E. and 8.13 % ± 0.0519 S.E. respectively. The difference between group I and II,

group I and III, and group II and III was significant (P=0.000 for all). The correlation (r) between hemoglobin A1c and serum IL-18 shown in the figure

2 was 0.641 with P-value 0.000 which is significant in type 2 diabetics (group II). The correlation (r) between serum creatinine and serum IL-18 shown in the figure 2 was 0.041 with P-value 0.830 which is not significant in group III.

Figure 1 showed that the correlation coefficient (r) between urinary albumin to creatinine ratio and IL-18 was 0.983 with P-value 0.000 which is significant in group III.

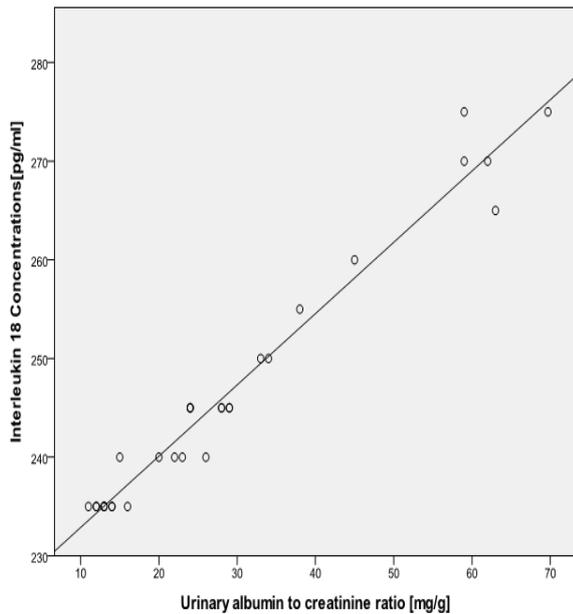


Figure 1: Correlation of serum IL-18 concentrations with urinary albumin to creatinine ratio group III.

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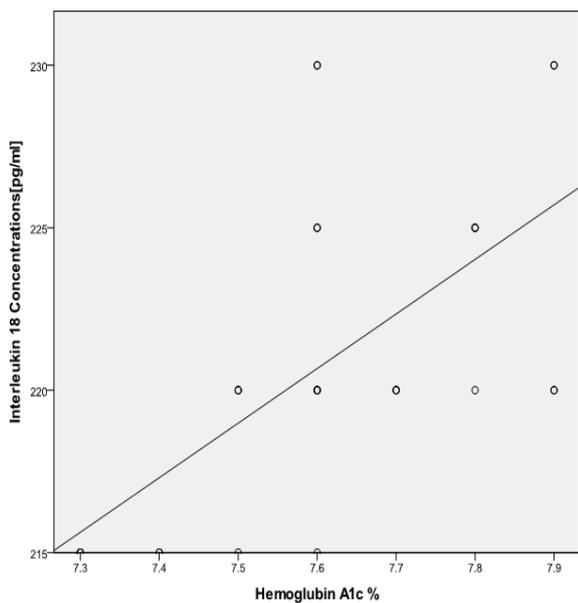


Figure 2. Correlation of Serum IL-18 Concentrations with HbA1c in group II.

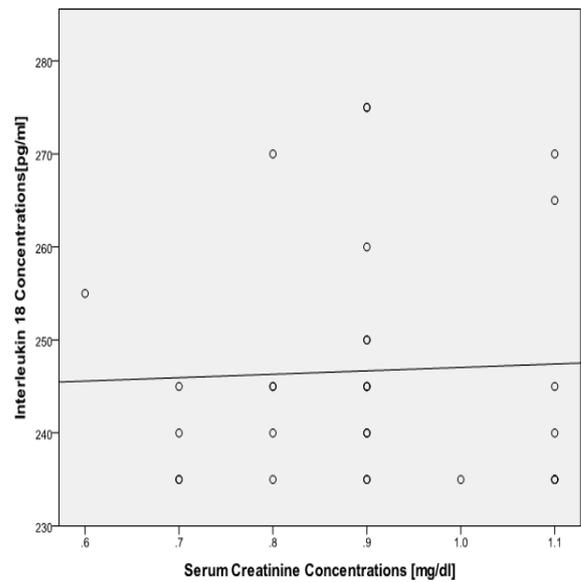


Figure 3: Correlation of Serum IL-18 Concentrations with Serum Creatinine in group III.

DISCUSSION

It is a cytokine named previously as an interferon gamma inducing factor (IFGI). It acts as an augmentation of interferon gamma (IFN - γ) production also as a developmental regulation of T helper 1 (TH1) as well as Fas – mediated cytotoxicity, so IL-18 can be a pleiotropic immunoregulatory factor [16].

There is an increase of IL-18 concentration in group II; moreover, IL-18 levels are increased in group III, this increase is proportional to the increase of UACR level. Correspondingly Moriwaki et al. [5] and Sahar and Eman [17] found that serum IL-18 levels are increased in diabetic patients with normoalbuminuria (urinary albumin to creatinine ratio <30 mg/g) in comparison with healthy control group; also they found that serum IL-18 levels are increased in diabetic patients with microalbuminuria (urinary albumin to creatinine ratio 30-300 mg/g) in comparison with diabetic patients with normoalbuminuria. In addition Nakamura et al. [18] concluded that serum IL-18 levels were significantly elevated in patients with type 2 diabetes as compared with control subjects. Likewise, Mahmoud et al. [19] noticed that the levels of IL-18 were significantly higher in both types of diabetics as compared to the control group. IL-18 levels were increased gradually in diabetics with nephropathy, and this increase was gradual reaching the highest level in microalbuminuric stage. The reason for the serum IL-18 elevation in group II was attributed to the effect of hyperglycemia [20]. Liang et al. [21] concluded that IL-18 receptors are ordinarily expressed in the proximal tubular epithelial cells (TEC), IL-18 receptors increase as long as the IL-18 increase in the chronic kidney disease. Finally Liang

et al. [21] concluded that IL-18 may play a role in promoting TEC injury and their activation which leads to renal tubulointerstitial fibrosis (TIF). The pleiotropic immunoregulatory roles of serum IL-18 are several such as promoting production of other inflammatory cytokines (including IL-1 and TNF- α) [22]. IL-18 secretes monocytes chemotacting protein-1 (MCP-1) and induces apoptosis [21]. IL-18 has a primary role in intracellular cell adhesion molecule-1 (ICAM-1) [23]. IL-18 induces interferon gamma (IFN- γ) production from T helper I cells that leads to tissue injury in inflammatory reaction [24].

The correlation ($r=0.983$) between the levels of urinary albumin to creatinine ratio (UACR) and serum IL-18 which are positively correlated in group III. The reason of this positive correlation back to hyperglycemia that induce inflammatory mediators such as IL-18 in renal tissue [23] and as IL-18 receptors are constitutively present in proximal renal tubules and increase with kidney tissue injury [21] simultaneously postulated with cubilin-megalyn receptors damage, so the microalbuminuria will increase as long as serum IL-18 increase. Also Nakamura et al. [18] found that there is a positive correlation between serum IL-18 and albumin excretion rate (AER) in the diabetic patients with nephropathy and then they suggested that IL-18 might be considered as a good predictor for DN. Similarly Mahmoud et al. [19] found that correlation analysis showed that the serum IL-18 concentration was positively correlated with urinary albumin levels; suggested that high serum levels of IL-18 might play a role in the pathogenesis of diabetes mellitus and in the development of nephropathy in diabetic patients of type 1 and type 2.

The present study revealed that there were no significant differences in serum creatinine levels between the studied groups. In the early stage of kidney involvement, serum creatinine detection was found to be deficient to detect this mild kidney impairment [25 & 26]. There is no correlation between serum creatinine and serum IL-18 in group III in the early stage of DN. The reason of this non significant correlation revert to the early stage of diabetic nephropathy, in this stage the glomerular filtration process in which serum creatinine infiltrated outside the body is intact, as serum IL-18 increase in this stage. Our results are agreed with Pagtalunan et al. [27] results that found serum creatinine level was at the normal level in early diabetes and in microalbuminuria. In the late stage they explained the cause of progression of diabetic nephropathy on the basis of podocyte loss and broadening of their foot processes since broadening of podocyte foot processes was associated with a reduction in the number of

podocytes per glomerulus and an increase of surface area covered by remaining podocytes, and as serum creatinine is removed by filtration through glomeruli of the kidney and is excreted into urine [28], so serum creatinine level still at the normal range in this early stage of diabetic nephropathy. Also Turkmen et al. [29] stated that spot urine IL-18 levels considered as an early parameter for identifying kidney diseases as it is increased within 6-24 hours after induction of acute kidney injury; beside that these researchers found that there was no significant correlation between spot urinary IL-18 and plasma creatinine.

There is a significant positive correlation ($r=0.641$, $P=0.000$) between serum IL-18 levels and HbA1c levels in group II. The reason of this positive correlation may back to effect of hyperglycemia upon increase of IL-18 production [20]. Mahmoud et al. and Dezayee [19 & 30] also found a positive correlation between serum IL-18 and HbA1c in type 2 diabetes mellitus. This positive correlation gives us a clear evidence that serum IL-18 can be considered as good predictors for diabetic nephropathy.

In conclusion, it could be suggested that IL-18 considered as a predictive factor for monitoring the early DN and also the possibility of having a remedial approach effect on DN development.

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