Serum allograft inflammatory factor-1 concentration in type 2 diabetes mellitus and its relation to the pathogenesis and progression of diabetic nephropathy

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Received 12 December 2015
Accepted 23 September 2016

Egyptian Journal of Obesity, Diabetes and Endocrinology
2016, April:7–17

Objective
Inflammatory mechanisms may play a pivotal role in diabetic nephropathy (DN). Allograft inflammatory factor-1 (AIF-1), a marker of activated macrophage, may have a role in the progression of DN.

Aim
The aim of the present study was to examine the relationship between serum AIF-1 concentration and parameters of DN.

Patients and methods
A total of 80 type 2 diabetes patients and 20 healthy volunteers (control group) were included in the present study. Patients with renal dysfunction or inflammatory conditions were excluded. Clinical and laboratory tests for patients and controls were carried out. The patients' group was classified according to the Urinary Albumin Excretion (UAE) level into the following: group IA (normoalbuminuria group), which included 30 patients with UAE less than 30 mg/g of creatinine (mg/g Cr); group IIA (microalbuminuria group), which comprised 25 patients with UAE from 30 to 300 mg/g Cr; and group IIIA (macroalbuminuria group), which included 25 patients with UAE greater than 300 mg/g Cr. All patients were subjected to further classification according to estimated glomerular filtration rate (eGFR) into the following: group IB, which included 31 patients with eGFR less than or equal to 60 ml/min/1.73 m^2; and group IIB, which included 49 patients with eGFR greater than 60 ml/min/1.73 m^2.

Results
AIF-1 was significantly raised in all patients compared with controls (P = 0.001), and in both group IIA and group IIIA than in group IA (P = 0.001). AIF-1 had significant positive correlation with age, diabetes duration, UAE, log urinary albumin creatinine (A/C) ratio, urea, creatinine, and Fasting Blood Sugar (FBS) (P < 0.001). AIF-1 concentration was inversely correlated with eGFR. Serum AIF-1 was significantly raised in group IB (112.35 ± 26.8) compared with group IIB (83.41 ± 26.23) (P < 0.001). Serum AIF-1 was significantly raised in both groups of simple and proliferative diabetic retinopathy than in the group of nondiabetic retinopathy (P = 0.001).

Conclusion
AIF-1 was significantly raised in type 2 diabetic patients and in those with DN and retinopathy, which may raise a possibility of their pathogenesis as an inflammatory process.

Keywords:
allograft inflammatory factor-1, albuminuria, diabetic nephropathy, retinopathy

Egyptian Journal of Obesity, Diabetes and Endocrinology April:7–17

2356-8062

Introduction

Recent studies have shown that chronic inflammation is associated with the development and progression of type 2 diabetes mellitus (DM), implying that immunologic and inflammatory mechanisms may play a pivotal role in the disease process. Furthermore, increased infiltration of monocytes/macrophages and activated T lymphocytes, as well as augmented expression of inflammatory cytokines in the kidneys, have also been found in patients with diabetic nephropathy (DN) [1–3].

Serum allograft inflammatory factor-1 (AIF-1) was originally cloned from activated macrophages in human and rat atherosclerotic allogenic heart grafts undergoing chronic transplant rejection [4]. Subsequently, there were reports of macrophages expressing AIF-1 in various diseases, such as macrophages in the pancreatic islets in prediabetic rats [5], in the human allograft kidney undergoing clinical rejection [6], in the brain of experimental autoimmune encephalomyelitis [7], and in the skeletal muscle after devascularization [8].

As regards the possible role of macrophages in DN and AIF-1 being a marker of activated macrophage, the current study aimed to study the relationship between serum AIF-1 concentration and parameters of DN, and whether AIF-1 might be considered as a novel biomarker to assess the progression of DN [i.e., to explore its association with the degree of urinary albumin excretion and estimated glomerular filtration rate (eGFR)].

Patients and methods

This prospective, cross-sectional study included 80 type 2 diabetic patients (patients' group) after obtaining an informed written consent. Patients were selected from those coming for follow-up at Minia University Hospital, Internal Medicine Outpatient Clinic (from December 2013 till December 2014). They were known to have type 2 DM according to the criteria proposed by the American Diabetic Association [9].

Control group

This study also included 30 healthy individuals of matched age and sex and free of any chronic medical diseases that may affect kidney functions.

Exclusion criteria

Patients with advanced renal dysfunction (serum creatinine ≥2.0 mg/dl) [10], patients with inflammatory conditions, or those on medications such as NSAIDs or steroids were excluded from the present study.

All patients were interviewed according to a standard questionnaire that covered clinical characteristics; smoking status (nonsmokers, ex-smokers, or current smokers); presence of cardiovascular disease (CVD) (defined as previous myocardial or cerebral infarction); and current medication of insulin, oral antidiabetic drugs, statins or angiotensin-converting enzyme inhibitors (ACEIs), and/or angiotensin II receptor blockers (ARBs).

(1) Systolic and diastolic blood pressures were measured after 5 min of rest
(2) BMI was calculated as proposed by the National Institute of Health (BMI = weight in kg/height in m^2). Obesity was defined as a BMI of greater than or equal to 30 kg/m^2 [11]
(3) As regards fundus examination, retinopathy was assessed by an ophthalmologist who was unaware of the data, and graded as follows:
    (a) Nondiabetic retinopathy (NDR)
    (b) Simple diabetic retinopathy (SDR)
    (c) Proliferative diabetic retinopathy (PDR) [12].

If the finding in the left and right fundi were discordant, the worse side was taken as a representative for the participant.

Laboratory investigations

A sample of 10 ml of venous blood after overnight fasting was obtained from all participants to estimate the following:

(1) Fasting blood sugar, serum urea, creatinine, total cholesterol, and triglyceride (TG) were assayed using the fully automated clinical chemistry autoanalyzer system Konelab 60i (Thermo Electron Incorporation, Helsinki, Finland)
(2) eGFR was calculated using CKD-EPI:

$$\begin{aligned}\mathrm{GFR\,(ml/min)} = {} & 141 \times \mathrm{minimum} \\ & \left(\mathrm{serum\,creatinin}/\kappa, 1\right)\alpha \\ & \times \mathrm{maximum} \\ & \left(\mathrm{serum\,creatinin}/\kappa, 1\right)^{-1.209} \\ & \times 0.993^{\mathrm{age}} \times \mathrm{sex} \times \mathrm{race}.\end{aligned}$$

For women, the following values are used: sex = 1.018; α = −0.329; κ = 0.7. For males, the following values are used: sex = 1; α = −0.411; κ = 0.9 [13]

(3) Hemoglobin A1c% (HbA1c) was assayed quantitatively by using boronate affinity by NycoCard Reader II (Alere/Axis-Shield, Oslo, Norway) [14]
(4) Serum AIF-1 was measured quantitatively by using the enzyme-linked immunosorbent assay [15]
(5) A/C ratio was determined in an early morning spot urine with an immune-turbidimetric assay [16].

Clinical study

Patients were classified according to UAE level into the following:

(1) Group IA (normoalbuminuria group), which included 30 patients with UAE less than 30 mg/g of creatinine (mg/g Cr)
(2) Group IIA (microalbuminuria group), which included 25 patients with UAE from 30–300 mg/g Cr
(3) Group IIIA (macroalbuminuria group), which included 25 patients with UAE greater than 300 mg/g Cr

Patients were subjected to another classification on the basis of the eGFR into the following [17]

(1) Group IB, which included 31 patients with eGFR less than or equal to 60 ml/min/1.73 m^2
(2) Group IIB, which included 49 patients with eGFR greater than 60 ml/min/1.73 m^2.

Statistical study

The collected data were tabulated and statistically analyzed using SPSS program software (version 20; SPSS Inc., Chicago, Illinois, USA). Numerical data were described by using mean ± SD and minimum and maximum of the range, whereas categorical data were described by using number and percentage. Analyses were carried out for quantitative variables using the independent sample *t*-test for parametric data between the two groups. The χ^2-test was used for qualitative data between groups. Correlation between two quantitative variables was established by using Pearson's correlation coefficient, and for nonparametric variables by using Spearman's ρ correlation test. Correlation coefficient ranged from 0 to 1: weak (r = 0–0.24), fair (r = 0.25–0.49), moderate (r = 0.5–0.74), and strong (r = 0.75–1). Cut-off value of AIF-1 was estimated to define the level at which albuminuria could be indirectly detected. Multiple linear regression analysis was carried out for estimating whether AIF-1 is an independent variable for predicting A/C ratio and eGFR. Level of significance was set at a *P* value of less than 0.05.

Results

The mean age increased significantly for patients with macroalbuminuria, with significant difference compared with other groups (P < 0.001 and <0.019, respectively) (Tables 1–3). There was a gradual increase in the duration of diabetes with increased severity of albuminuria, with significant difference between all groups (P < 0.001). Group IIIA comprised 14 (56%) patients with hypertension, with a significant difference with other groups (P < 0.01). This group (group IIIA) showed significant long duration of hypertension and increased prevalence of ACE intake compared with other groups (P < 0.05). As regards fundus examination, group IA included 96.7% patients with NDR and one (3.3%) patient with SDR. Group IIA included 84% patients with NDR, 3.3% patients with SDR, and one (4%) patient with PDR, whereas group IIIA included 52% patients with NDR, 32% patients with SDR, and 16% patients with PDR. There was a significant difference between all groups (P < 0.002).

Table 4 reveals that fasting blood glucose was raised in group IIIA, with a significant difference between this group and group IA (P < 0.01). Regarding serum creatinine, there was a significant difference between all groups (P < 0.001). eGFR and HbA1c showed significant difference between all groups (P < 0.001). Fig. 1 shows that, in group I, AIF-1 showed significant difference between group I and III (P < 0.001), between group II and III (P < 0.001), and between all groups (P < 0.001).

Table 1 Comparative study between patients and controls as regards demographic and clinical data

Variables	Patients (*n*=80)	Control (*n*=30)	*P* value
Age (years)			
Range	43-77	41-73	0.189
Mean±SD	56.92±8.54	66.3±4.14	
Sex (*n* (%))			
Male	38 (47.5)	15 (50)	0.815
Female	42 (52.5)	15 (50)	
BMI (kg/m^2)			
Range	18-49	19-26	<0.001*
Mean±SD	31.32±6.34	22.45±2.18	
Smoking (*n* (%))			
No	56 (70)	30 (100)	0.003*
Yes	9 (11.2)	0 (0)	
Ex-smoker	15 (18.8)	0 (0)	
DM (*n* (%))			
No	52 (65)	30 (100)	<0.001*
Yes	28 (35)	0 (0)	
DM treatment (*n* (%))			
None	0 (0)	30 (100)	<0.001*
Oral	48 (60)	0 (0)	
Insulin	32 (40)	0 (0)	
HTN (*n* (%))			
No	52 (65)	30 (100)	<0.001*
Yes	28 (35)	0 (0)	
SBP (mmHg)			
Range	100-160	90-130	<0.001*
Mean±SD	125.18±17.14	112.5±12.3	
DBP (mmHg)			
Range	60-100	60-80	0.002*
Mean±SD	76.81±12.12	70±9.09	
History of ACEI intake (*n* (%))			
No	59 (73.8)	30 (100)	0.002*
Yes	21 (26.2)	0 (0)	
History of CVD (*n* (%))			
No	70 (87.5)	30 (100)	0.042*
Yes	10 (12.5)	0 (0)	
History of statins intake (*n* (%))			
No	56 (70)	30 (100)	0.001*
Yes	24 (30)	0 (0)	

ACEI, angiotensin-converting enzyme inhibitors; CVD, cardiovascular disease; DBP, diastolic blood pressure; DM, diabetes mellitus; HTN, hypertension; SBP, systolic blood pressure.

Table 5 shows that AIF-1 had significant positive correlation with age, diabetes duration, UAE, log A/C ratio, urea and creatinine, FBS, with *P* less than 0.001 for all of them except for age (P < 0.006) and FBS (P < 0.03), whereas AIF-1 concentration inversely correlated with eGFR. Table 6 shows the patients' group classified according to their eGFR level into two groups: group IB, which included 31 patients with eGFR less than or equal to 60 ml/min/1.73 m^2; and group IIB, which included 49 patients with eGFR greater than 60 ml/min/1.73 m^2.

The mean age and duration of diabetes increased significantly in patients with eGFR less than 60

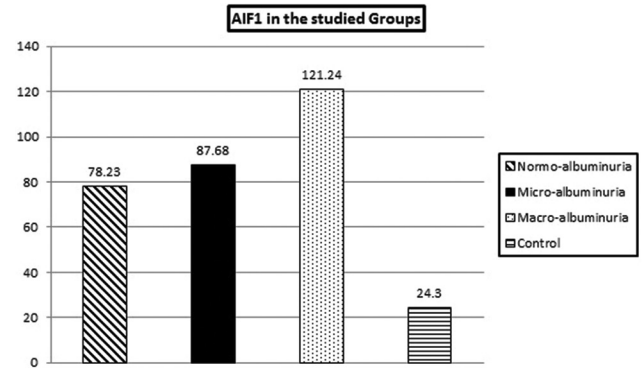
compared with other groups (P < 0.001 and <0.03, respectively). There was a significant increase in the prevalence of hypertensive patients with lower eGFR (P < 0.001). As regards the history of ACEI/ARBs drugs intake, there was a significant difference between the two groups (P < 0.001).

Table 2 Comparative study between patients and controls as regards laboratory results

Variables	Patients (*n*=80)	Control (*n*=30)	*P* value
TC (mg/dl)			
Range	53-300	100-190	>0.001*
Mean±SD	171.73±55.25	129.8±24.43	
TG (mg/dl)			
Range	43-388	85-124	0.007*
Mean±SD	115.27±44.88	99.8±13.36	
FBS (mg/dl)			
Range	101-450	89-140	>0.001*
Mean±SD	213.36±61.81	112.3±16.97	
HbA1c (%)			
Range	6.5-8.9	4.5-6.2	>0.001*
Mean±SD	7.29±0.61	5.42±0.58	
Fundus examination (*n* (%))			
NDR	63 (78.8)	30 (100)	0.023*
SDR	12 (15)	0 (0)	
PDR	5 (6.2)	0 (0)	
Blood urea (mg/dl)			
Range	15-180	22-35	0.004*
Mean±SD	47.86±33.96	29.4±3.67	
Serum creatinine (mg/dl)			
Range	0.4-2	0.6-0.9	>0.001*
Mean±SD	1.07±0.39	0.75±0.09	
eGFR (ml/min/1.73 m^2)			
Range	28-137	90-125	>0.001*
Mean±SD	72.55±25.82	101.8±11.11	
AIF-1 (pg/ml)			
Range	45-170	15-40	>0.001*
Mean±SD	94.62±29.87	24.3±8.41	

AIF-1, allograft inflammatory factor-1; eGFR, estimated glomerular filtration rate; HbA1c, hemoglobin A1c; NDR, nondiabetic retinopathy; PDR, proliferative diabetic retinopathy; SDR, simple diabetic retinopathy; TC, total cholesterol; TG, triglyceride.

Figure 1



Allograft inflammatory factor-1 (AIF-1) in patients' groups classified according to albuminuria.

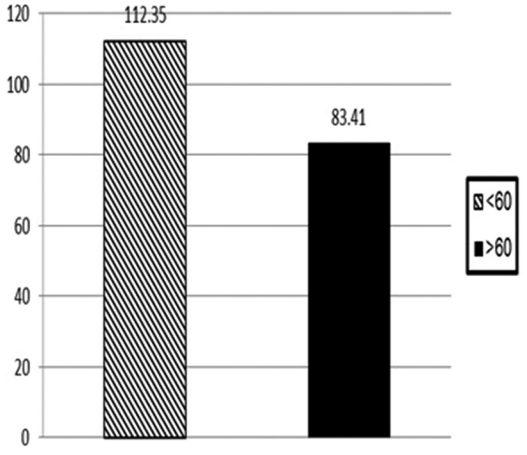
As regards fundus examination, group IB included 58.1% patients with NDR, 29% patients with SDR, and 12.9% patients with PDR. Whereas group IIB included 91.84% patients with NDR, 6.12% patients with SDR, and one (2.04%) patient with PDR. There was a significant difference between all groups (P < 0.002). Table 7 shows that UAE ratio and HbA1c both showed significant difference between both groups (P < 0.001). Serum AIF-1 in group IB ranged between 70 and 168 pg/ml (112.35 ± 26.8), whereas in group IIB it ranged from 45 to 170 pg/ml (83.41 ± 26.23), with a significant difference between the two groups (P < 0.001) (Fig. 2).

Table 8 and Fig. 3 show that serum AIF-1 was significantly raised in group III with PDR, with a significant difference between this group and group I (NDR) (P < 0.001) (Fig. 4 and Tables 9–12).

Discussion

In contrast to type 1 DM, type 2 DM is a nonautoimmune form of DM characterized by insulin resistance and relative (rather than absolute) insulin deficiency [18]. Macrophage accumulation is a feature of type 2 DM and is associated with the development of diabetic complications (nephropathy, atherosclerosis, neuropathy, and retinopathy) [19,20]. Albuminuria serves as a tool for monitoring nephron injury and as a response to therapy in DN. Microalbuminuria may be less predictive of DN and progression to macroalbuminuria in type 2 DM, as it may be secondary to factors unrelated to DM, such as hypertension, congestive heart failure, prostate disease, or infection [21].

Figure 2



Comparative study of serum allograft inflammatory factor-1 in group IB with estimated glomerular filtration rate (eGFR) less than 60 ml/min/1.73 m^2 and group IIB with eGFR more than 60 ml/min/1.73 m^2.

Table 3 Comparative study of the demographic and the clinical data of group IA, group IIA, and group IIIA

Variables	Group IA (normoalbuminuria) (*n*=30)	Group IIA (microalbuminuria) (*n*=25)	Group IIIA (macroalbuminuria) (*n*=25)	*P* value		
Age (years)					0.001*	
Range	43-69	46-72	47-77	I vs. II	I vs. III	II vs. III
Mean±SD	53.83±6.61	55.68±7.04	61.88±9.94	0.666	0.001*	0.019*
Sex (*n* (%))					0.310	
Male	11 (36.7)	13 (52)	14 (56)	I vs. II	I vs. III	II vs. III
Female	19 (63.3)	12 (48)	11 (44)	0.254	0.152	0.777
BMI (kg/m^2)					0.139	
Range	22-49	23-45	18-37	I vs. II	I vs. III	II vs. III
Mean±SD	32.16±6.64	32.4±6.98	29.24±4.88	0.990	0.203	0.182
Smoking (*n* (%))					0.917	
No	21 (70)	18 (72)	17 (68)	I vs. II	I vs. III	II vs. III
Yes	3 (10)	2 (8)	4 (16)	0.967	0.774	0.668
Ex-smoker	6 (20)	5 (20)	4 (16)			
Diabetes duration (years)					>0.001*	
Range	1-7	3-8	7-28	I vs. II	I vs. III	II vs. III
Mean±SD	3.33±1.34	5.48±0.96	12.44±5.18	0.030*	>0.001*	>0.001*
DM treatment (*n* (%))					0.087	
Oral	22 (73.3)	11 (44)	15 (60)	I vs. II	I vs. III	II vs. III
Insulin	8 (26.7)	14 (56)	10 (40)	0.027*	0.294	0.258
HTN (*n* (%))					0.010*	
No	25 (83.3)	16 (64)	11 (44)	I vs. II	I vs. III	II vs. III
Yes	5 (16.7)	9 (36)	14 (56)	0.101	0.002*	0.156
HTN duration					0.124	
Range	2-20	2-15	1-15	I vs. II	I vs. III	II vs. III
Mean±SD	7.2±7.32	5.38±4.13	9.85±4.55	0.793	0.570	0.111
SBP (mmHg)					0.664	
Range	110-160	100-155	100-160	I vs. II	I vs. III	II vs. III
Mean±SD	123.2±13.98	125.4±17.07	127.4±20.72	0.882	0.639	0.912
DBP (mmHg)					0.710	
Range	60-100	60-100	60-100	I vs. II	I vs. III	II vs. III
Mean±SD	76.5±10.09	75.6±12.27	78.4±14.34	0.960	0.834	0.698
History of ACEI					0.050*	
No	25 (83.3)	20 (80)	14 (56)	I vs. II	I vs. II	I vs. II
Yes	5 (16.7)	5 (20)	11 (44)	0.750	0.026*	0.069
History of CVD (*n* (%))					0.624	
No	25 (83.3)	22 (88)	23 (92)	I vs. II	I vs. III	II vs. III
Yes	5 (16.7)	3 (12)	2 (8)	0.625	0.337	0.637
History of statins (*n* (%))					0.574	
No	23 (76.7)	16 (64)	17 (68)	I vs. II	I vs. III	II vs. III
Yes	7 (23.3)	9 (36)	8 (32)	0.303	0.472	0.765
Fundus examination (*n* (%))					0.002*	
NDR	29 (96.7)	21 (84)	13 (52)	I vs. II	I vs. III	II vs. III
SDR	1 (3.3)	3 (12)	8 (32)	0.241	>0.001*	0.051
PDR	0 (0)	1 (4)	4 (16)			

ACEI, angiotensin-converting enzyme inhibitors; CVD, cardiovascular disease; DBP, diastolic blood pressure; DM, diabetes mellitus; HTN, hypertension; NDR, nondiabetic retinopathy; PDR , proliferative diabetic retinopathy; SBP, systolic blood pressure; SDR, simple diabetic retinopathy.

In the current study, no significant difference was found between the groups as regards sex. This is in agreement with a study conducted by Fukui *et al.* [22], who reported that the UAE was not affected by gender. In contrast, a study by Furtner *et al.* [18] reported significant difference between UAE in female versus male patients, and a study by Valmadrid *et al.* [23] reported that the patients with increasing levels of proteinuria were older, more likely to be male, and were to be maintained on insulin because of side effects of oral hypoglycemic drugs.

In the present study, the mean age increased significantly in patients with macroalbuminuria compared with other groups. In their study, Furtner *et al.* [18] reported that UAE was significantly related to age and reflected

Table 4 Comparative study of the laboratory data of group IA, group IIA, and group IIIA

Variables	Group IA (normoalbuminuria) (*n*=30)	Group IIA (microalbuminuria) (*n*=25)	Group IIIA (macroalbuminuria) (*n*=25)	*P* value		
FBS (mg/dl)					0.720	
Range	115-450	101-400	180-363	I vs. II	I vs. III	I vs. III
Mean±SD	190.36±63.91	215.92±62.06	238.4±49.68	0.255	0.010*	0.377
TG (mg/dl)					0.472	
Range	56-388	43-212	70-180	I vs. II	I vs. III	II vs. III
Mean±SD	120.33±61.11	106.16±36.25	118.32±25.97	0.479	0.985	0.607
TC (mg/dl)					0.720	
Range	53-260	71-300	71-250	I vs. II	I vs. III	II vs. III
Mean±SD	172.93±52.45	177.32±64.07	164.72±50.33	0.955	0.850	0.705
Urea (mg/dl)					0.006*	
Range	15-75	17-133	27-180	I vs. II	I vs. III	II vs. III
Mean±SD	36.06±13.91	45.44±31.81	64.44±45.65	0.533	0.005*	0.100
Creatinine (mg/dl)					>0.001*	
Range	0.5-1.8	0.4-2	0.8-1.8	I vs. II	I vs. III	II vs. III
Mean±SD	0.87±0.29	1.06±0.44	1.32±0.31	0.127	>0.001*	0.026*
eGFR (ml/min/1.73 m^2)					>0.001*	
Range	38-114	28-137	30-89	I vs. II	I vs. III	II vs. III
Mean±SD	85.1±21.25	74.88±29.88	55.16±15.43	0.231	>0.001*	>0.009*
UAE ratio (mg/g)					>0.001*	
Range	10.5-28	35-300	450-9000	I vs. II	I vs. III	II vs. III
Mean±SD	19.51±5.09	177±79.45	2471.5±2718.2	>0.001*	>0.001*	>0.001*
HbA1c (%)					>0.001*	
Range	6.5-7.6	6.6-8	6.6-8.9	I vs. II	I vs. III	II vs. III
Mean±SD	6.94±0.28	7.14±0.43	7.86±0.64	0.243	>0.001*	>0.001*
AIF-1 (pg/ml)					<0.001*	
Range	46-108	45-126	85-170	I vs. II	I vs. III	II vs. III
Mean±SD	78.23±19.19	87.68±24.55	121.24±27.7	0.231	<0.001*	<0.001*

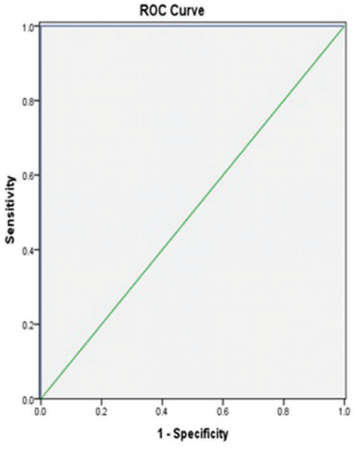
AIF-1, allograft inflammatory factor-1; eGFR, estimated glomerular filtration rate; HbA1c, Hemoglobin A1c; TC, total cholesterol; TG, triglyceride.

Table 5 Correlation between allograft inflammatory factor-1 and other variables in patients as a whole group

	AIF-1	
	r	*P* value
Age	0.303	0.006*
Diabetes duration	0.424	<0.001*
BMI	–0.064	0.571
TC	–0.077	0.497
TG	0.008	0.945
SBP	0.168	0.135
DBP	0.118	0.295
eGFR	–0.486	<0.001*
UAE ratio	0.659	<0.001*
Log A/C ratio	0.769	<0.001*
FBG	0.332	0.003*
Urea	0.413	<0.001*
Creatinine	0.480	<0.001*

A/C ratio, albumin/creatinine ratio; AIF-1, allograft inflammatory factor-1; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; FBG, fasting blood glucose; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride.

Figure 3



Receiver operating characteristic curve analysis for sensitivity and specificity of allograft inflammatory factor-1 in the prediction of albuminuria.

systemic endothelial leakiness. It may arise from an interaction between noxious influences of vascular risk attributes and a predisposing genetic background.

As regards smoking habits and BMI, there was an insignificant difference between the studied groups. This is in agreement with the results of a study by Furtner *et al.* [18], who found that UAE was not affected by BMI or smoking.

On the other hand, Fukui *et al.* [22] suggested that obesity is associated with low-grade chronic inflammation that is characterized by an inflamed

Table 6 Comparative study between group IB and group IIB as regards demographic data

	Group IB eGFR ≤60 (*n*=31)	Group IIB eGFR >60 (*n*=49)	*P* value
Age (years)			
Range	47-77	43-76	<0.001*
Mean±SD	61.25±9.35	54.18±6.75	
Sex (*n* (%))			
Male	18 (58.1)	20 (40.8)	0.132
Female	13 (41.9)	29 (59.2)	
BMI (kg/m^2)			
Range	18-45	22-49	0.234
Mean±SD	30.25±6.55	32±6.18	
Smoking (*n* (%))			
No	22 (71)	34 (69.4)	0.854
Yes	4 (12.9)	5 (10.2)	
Ex-smoker	5 (16.1)	10 (20.4)	
DM duration (years)			
Range	2-28	1-18	<0.001*
Mean±SD	9.8±5.98	4.97±2.89	
DM treatment (*n* (%))			
Oral	14 (45.2)	34 (69.4)	0.031*
Insulin	17 (54.8)	15 (30.6)	
HTN (*n* (%))			
No	13 (41.9)	39 (79.6)	0.001*
Yes	18 (58.1)	10 (20.4)	
HTN duration (years)			
Range	1-15	2-20	0.249
Mean±SD	8.81±4.91	6.4±5.64	
SBP (mmHg)			
Range	100-160	100-160	0.053
Mean±SD	129.83±18.95	122.24±15.37	
DBP (mmHg)			
Range	60-100	60-100	0.039*
Mean±SD	80.32±12.77	74.59±11.26	
History of ACEI intake (*n* (%))			
No	16 (51.6)	43 (87.8)	<0.001*
Yes	15 (48.4)	6 (12.2)	
History of CVD (*n* (%))			
No	25 (80.6)	45 (91.8)	0.140
Yes	6 (19.4)	4 (8.2)	
History of statins intake (*n* (%))			
No	20 (64.5)	36 (73.5)	0.395
Yes	11 (35.5)	13 (26.5)	
Fundus examination (*n* (%))			
NDR	18 (58.1)	45 (91.84)	0.002*
SDR	9 (29)	3 (6.12)	
PDR	4 (12.9)	1 (2.04)	

ACEI, angiotensin-converting enzyme inhibitor; CVD, cardiovascular disease; DBP, diastolic blood pressure; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; HTN, hypertension; NDR, nondiabetic retinopathy; PDR, proliferative diabetic Retinopathy; SBP, systolic blood pressure; SDR, simple diabetic retinopathy.

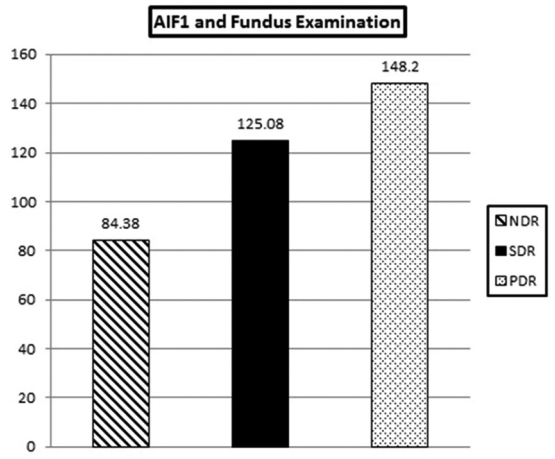
adipose tissue with increased infiltration of macrophages and accumulation of other immune cells, such as T cells, which may have a role in the pathogenesis of UAE in diabetes and possibly in of CVD [24,25].

Table 7 Comparative study of the laboratory data of group IB and group IIB

Variables	Group IB eGFR ≤60 (*n*=31)	Group IIB eGFR >60 (*n*=49)	*P* value
RBS (mg/dl)			
Range	140-363	101-450	0.321
Mean±SD	221.19±43.31	208.41±71.07	
TG (mg/dl)			
Range	59-180	43-388	0.611
Mean±SD	118.51±25.99	113.22±53.68	
TC (mg/dl)			
Range	71-280	53-300	0.446
Mean±SD	165.77±54.08	175.51±56.21	
Urea (mg/dl)			
Range	30-180	15-175	<0.001*
Mean±SD	67.87±38.91	35.2±23.01	
Creatinine (mg/dl)			
Range	1-2	0.4-1.4	<0.001*
Mean±SD	1.46±0.26	0.83±0.22	
UAE ratio (mg/g Cr)			
Range	20-9000	10.5-5000	<0.001*
Mean±SD	1681.38±2604.47	299.5±859.46	
AIF-1 (pg/ml)			
Range	70-168	45-170	<0.001*
Mean±SD	112.35±26.81	83.41±26.23	

AIF-1, allograft inflammatory factor-1; Cr, creatinine; eGFR, estimated glomerular filtration rate; RBG, random blood glucose; TC, total cholesterol; TG, triglyceride.

Figure 4



Serum allograft inflammatory factor-1 (AIF-1) and fundus examination. NDR, nondiabetic retinopathy; PDR, proliferative diabetic retinopathy; SDR, simple diabetic retinopathy.

Activated macrophages in the adipose tissue, besides contributing to insulin resistance [26], may also be directly involved in the regulation of fat mass [25]. Adipose tissue macrophages produce a number of proinflammatory cytokines that promote adipose dysfunction and insulin resistance [27].

In their respective studies, Thorleifsson *et al.* [28], Casimiro [29], and Lorente-Cebrián *et al.* [30] found that AIF-1 was secreted in a time-dependent

Table 8 Sensitivity and specificity of allograft inflammatory factor-1 in the diagnosis of diabetic nephropathy

	Cutoff point	AUC	*P* value	Sensitivity	Specificity	PPV	NPV	Accuracy
AIF-1	>40	1	<0.001*	100%	100%	100%	100%	100%

AUC, area under the curve; AIF-1, allograft inflammatory factor-1; NPV, negative predictive value; PPV, positive predictive value.

Table 9 Evaluation of serum allograft inflammatory factor-1 concentration as an independent determinant of estimated glomerular filtration rate by multivariate linear regression analysis after adjustment for the following variables: duration of diabetes, body mass index, hemoglobin A1c%, systolic blood pressure, total cholesterol, triglyceride

	B	*t*	*P* value
Constant	108.01	2.585	<0.001*
AIF-1	–0.359	–3.002	0.004*

Estimated glomerular filtration rate=108.01+(–0.359×AIF-1).
AIF-1, allograft inflammatory factor-1.

Table 10 Evaluation of serum allograft inflammatory factor-1 concentration as an independent determinant of log urinary albumin creatinine ratio by multivariate linear regression analysis after adjustment for the following variables: duration of diabetes, body mass index, hemoglobin A1c%, systolic blood pressure, total cholesterol, triglyceride

	B	*t*	*P* value
Constant	–0.574	–0.682	0.497
AIF-1	0.015	6.227	<0.001*

Log urinary albumin creatinine ratio=–0.574+(0.015×AIF-1).
AIF-1, allograft inflammatory factor-1.

Table 11 Evaluation of serum allograft inflammatory factor-1 concentration as an independent determinant of urinary albumin creatinine ratio by multivariate linear regression analysis after adjustment for the following variables: duration of diabetes, body mass index, hemoglobin A1c, systolic blood pressure, total cholesterol, triglyceride

	B	*t*	*P* value
Constant	–7099.14	–2.783	0.007*
AIF-1	31.162	4.264	<0.001*

Urinary albumin creatinine ratio=–7099.14+(31.162×AIF-1).
AIF-1, allograft inflammatory factor-1.

fashion from the white adipose tissue from resident macrophages. They also observed that expression of AIF-1 was similar in visceral and subcutaneous white adipose tissue and there was a two-fold increase in obese women, and its levels were normalized after weight reduction. They also found that expression of AIF-1 was inversely correlated with insulin sensitivity as assessed by using the insulin tolerance test, and circulating levels of adiponectin, and positively correlated with insulin resistance as estimated by homeostasis model assessment for insulin resistance (HOMA-IR).

As regards the duration of diabetes, there was a significant difference between all groups ($P < 0.001$). Large cohort studies by Valmadrid *et al.* [23] and Fukui *et al.* [22] reported that UAE was positively correlated with the duration of diabetes, and the duration of diabetes was independently correlated with log UAE.

As DN progresses, there is a progressive increase in UAE and diminished renal function, which results from the thickening of the pathological basement membrane, atrophy, and interstitial fibrosis [1]. During the first 5 years of DM, thickening of the glomerular basement membrane, glomerular hypertrophy, and mesangial volume expansion occur as the GFR returns to normal [1]. After 5–10 years of DM, many individuals begin to excrete small amounts of albumin with urine [31].

A study by Park *et al.* [17] suggested that proteinuria was a more important indicator for DR than was decreased eGFR due to endothelial leakiness.

HbA1c showed significant difference between group IA and group IIIA ($P < 0.01$). Furtner *et al.* [18], in their study, stated that impaired glycemic control is associated with increased microvascular complication. Hyperglycemia induces macrophage production of IL-12, which stimulates CD-4 cell production of IFN-γ, and activates nuclear factor-κB through Protein Kinase C (PKC) and reactive oxygen species to rapidly stimulate the expression of cytokines [32]. Furthermore, longer disease duration results in increased advanced glycosylation end (AGE) products and AGE-modified proteins, which could bind to the receptor for AGE on macrophages and T cells, stimulating synthesis and release of proinflammatory cytokines in DM [2,29].

Intensive glycemic control was associated with significantly decreased rates of DN in patients with type 2 DM. Glucotoxicity and lipotoxicity cause synergistic effects on the development and progression of DN. Macrophages have emerged as potential contributors in mediating glucolipotoxicity through the activation of MRP8/toll-like receptor 4 (TLR4) signaling in diabetic glomeruli [33].

As regards the presence of hypertension, there was a significant difference between all groups ($P < 0.010$). This is in agreement with studies conducted by Furtner *et al.* [18] and Fukui *et al.* [22], in which there was a positive correlation between hypertension and UAE, which can be attributed to endothelial dysfunction and high intraglomerular pressure, which cause increased albumin excretion. However, a subset of patients with type 2 DM developed chronic kidney disease without nephrotic-range proteinuria. Whether this difference represents a fundamental difference in the

Table 12 Comparative study of serum allograft inflammatory factor-1 as regards fundal examination

	Fundus examination			*P* value		
	Group I (NDR) (*n*=30)	Group II (SDR) (*n*=25)	Group III (PDR) (*n*=25)			
AIF-1					<0.001*	
Range	45-150	91-168	126-170	I vs. II	I vs. III	II vs. III
Mean±SD	84.38±21.91	125.08±24.34	148.2±18.61	<0.001*	<0.001*	0.152

AIF-1, allograft inflammatory factor-1; NDR, nondiabetic retinopathy; PDR, proliferative diabetic retinopathy; SDR, simple diabetic retinopathy.

pathophysiology of the two conditions or represents the synergistic effects of other kidney injuries, such as hypertensive renal disease, is unclear [21]. Hypertension is usually absent in the early stages in patients with type 1 DM but is present in 10–25% of the patients with type 2 DM at their initial evaluations. Microalbuminuria is a more specific sign of DN in type 1 DM than in type 2 DM because of the high incidence of hypertension, which itself may lead to microalbuminuria in the latter [21].

Increased plasma prorenin activity was noted as a risk factor for the development of DN. Prorenin binds to a specific tissue receptor that promotes activation of mitogen-activating protein kinases (MAPK) [34].

Furthermore, activated renin–angiotensin–aldosterone system and endothelial dysfunction have been proven to be crucial determinants of leukocyte activation and cytokine expression in generating proinflammatory and proliferative effects [13,35].

Early identification of patients with DN allows for the intensification of the therapy, which slows the progression of kidney disease and helps in the management of the increased risk for CVD [36].

Hyperlipidemia represents an independent metabolic risk factor for the progression of DN. Its molecular mechanism involves TLR4 interacting with its potent ligand S100 calcium-binding protein A8 (calgranulin-A; S100A8) in macrophages, infiltrating the glomeruli of DN patients [37].

Statins were found to either prevent, delay, or even reverse the decline of GFR, as well as to reduce albuminuria in patients with type 2 DM [38], with CVD risk reduction in patients with CKD [in the Study of Heart and Renal Protection (SHARP)] [33,39].

As regards TGs and total cholesterol, no significant difference was found in the studied groups. In contrast, a study by Fukui *et al.* [22] reported an independent correlation between serum total lipids and log UAE. This may be due to the fact that most of our patients took statins for a short period of time and/or postponed taking statins until late.

Serum AIF-1 revealed significant difference between all groups ($P < 0.001$) with significant positive correlation with diabetes duration, UAE, log A/C ratio, urea and creatinine ($P < 0.001$), age ($P < 0.006$), and FBS ($P < 0.03$), whereas it showed inverse correlation with eGFR ($P < 0.001$).

This is in agreement with a study by Fukui *et al.* [22], who revealed that serum AIF-1 levels were higher in patients with macroalbuminuria than in those with normoalbuminuria ($P = 0.0001$) or with microalbuminuria ($P = 0.009$). Serum AIF-1 concentrations positively correlated with age and log UAE, whereas inversely correlated with eGFR. In addition, they found that serum AIF-1 levels positively correlated with the levels of FBS ($P = 0.006$), HbA1c% ($P = 0.003$), TG ($P = 0.02$), and BMI ($P = 0.001$), and inversely correlated with high-density lipoprotein ($P = 0.002$). Stepwise multiple regression analysis indicated that HbA1c% ($\beta = 0.133$, $F = 5.490$, $P < 0.05$) and waist circumference ($\beta = 0.197$, $F = 11.954$, $P < 0.05$) were independent predictors of serum AIF-1 levels. They suggested that AIF-1 plays an additional role in the dysfunction of β cells and may be considered as an early marker for DN and a significant predictor of activated macrophages, as well as CVD in humans [22].

In the current study, mean serum AIF-1 concentration was higher in patients with PDR ($P < 0.001$) than in patients with NDR, and in patients with SDR than in patients with NDR ($P < 0.001$). These results are in agreement with a theory proposed by Wu *et al.* [40], who demonstrated that macrophages were prominent in sections from diabetic patients with advanced diabetic retinopathy.

The current study showed that the mean age and duration of diabetes increased significantly in patients with eGFR less than or equal to 60 ($P < 0.001$). No significant difference was found between the two groups as regards gender. This is in agreement with a study by Park *et al.* [17]. Sex hormones may influence hyperfiltration, as Cherney *et al.* [41] in their study observed a decrease in the renal blood flow and vascular resistance in response to hyperglycemia in women, but not in men. The same study [41] showed that the addition of ACEI resulted in a decrease in the blood pressure in both men and women, but GFR decreased only in women.

As regards the BMI, there was no significant difference between the two groups. This was in agreement with the results obtained in a study by Park *et al.* [17], who found a insignificant association between BMI and eGFR.

As regards fundus examination, there was a significant difference between all groups ($P = 0.002$). These results are in agreement with Park *et al.* [17], who suggested that eGFR is an indicator for DR but not as significant as proteinuria due to endothelial leakiness.

The mean serum AIF-1 concentration was significantly higher in patients whose eGFR was less than or equal to 60 ml/min/1.73 m^2 compared with the other groups ($P < 0.001$). It correlated with log UAE and eGFR even after adjusting for the duration of diabetes, BMI, HbA1c, systolic blood pressure, serum total cholesterol, and TGs. This is in agreement with a study by Fukui *et al.* [22], who found that the serum AIF-1 concentration was higher in patients whose eGFR was less than 60 ml/min/1.73 m^2 compared with patients whose eGFR was greater than 90 ml/min/1.73 m^2 ($P = 0.002$) or with patients whose eGFR was between 60 and 90 ml/min/1.73 m^2 ($P = 0.007$).

They found that the systolic blood pressure, duration of diabetes, serum total cholesterol, TG, and AIF-1 concentrations were independently correlated with log UAE and the duration of diabetes. Furthermore, HbA1c% and serum TG and AIF-1 concentrations were independently correlated with eGFR. AIF-1 levels in healthy humans have been found to be positively correlated with metabolic indicators, such as BMI, TGs, and FBS [22].

Activated renin–angiotensin–aldosterone system proved to be a crucial determinant of leukocyte activation and cytokine expression in generating proinflammatory and proliferative effects [35]. AIF-1 protein is not expressed in quiescent cultured human Vascular smooth muscle cells (VSMCs) but is induced in cells challenged with various inflammatory cytokines, primarily by INF-γ, IL-1β, and T-cell-conditioned media [42].

Overexpression of AIF-1 in human VSMCs results in enhanced growth of these cells. This cytokine-induced activation and proliferation of medial VSMCs lead to intimal hyperplasia, the most critical cellular event in the formation of arteriosclerosis [43].

In their study, Chen *et al.* [4] proved that AIF-1 enhances VSMC growth by autocrine production of Granulocyte colony stimulating factor (G-CSF), and AIF-1-transduced VSMCs are chemotactic for human monocytes. Thus, its expression may influence VSMC–inflammatory cell communication.

Tian *et al.* [44] and Mishima *et al.* [45] found that the stimulation of human macrophages with oxidized low-density lipoprotein significantly increased AIF-1 expression above basal levels. They suggested a tight association between AIF-1 expression and macrophage activation. These data indicate that AIF-1 mediates atherogenesis-initiated signaling and activation of macrophages.

In their study, Tian *et al.* [44] found the following. First, AIF-1 is detected in Endothelial cells (EC) within the intima if inflamed human arteries and its expression can be induced in cultured EC by inflammatory and angiogenic factors. Second, knock-down of AIF-1 protein by stable transfection of siRNA reduces the several indices of EC pathophysiology, including proliferation and migration. These functions could be rescued by the exogenous expression of AIF-1. Third, signal transduction cascades could be reduced by AIF-1 abrogation. Fourth, although angiogenesis assays were not negatively affected by a reduction in AIF-1, angiogenic potential of EC was enhanced by AIF-1 overexpression.

Future strategies

The use of immunosuppressants and neutralizing antibodies may have a role in reducing leukocyte accumulation, inhibiting renal macrophage recruitment, and hence suppressing the development of renal injury. Future studies are needed to evaluate the anti-inflammatory strategies to demonstrate antiproteinuric and renoprotective effects [2]. Macrophage migration inhibitory factor, which is a proinflammatory cytokine produced by both immune and nonimmune cells, may be a potential therapeutic strategy for DN [46].

Acknowledgements

The authors thank the Faculty of Medicine, Minia University, for the financial support of this study.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1 Tervaert TW, Mooyaart AL, Amann K, Cohen AH, Cook HT. Renal Pathology Society. Pathologic classification of diabetic nephropathy. J Am Soc Nephrol 2010; 21:556-563.

2 Wu CC, Sytwu HK, Lin YF. Cytokines in diabetic nephropathy. Adv Clin Chem 2012; 56:55–74.

3 Wu HY, Huang JW, Lin HJ, Liao WC. Comparative effectiveness of renin–angiotensin system blockers and other antihypertensive drugs in patients with diabetes: systematic review and Bayesian network meta-analysis. BMJ 2013; 347:f 6008.

4 Chen X, Kelemen SE, Autieri MV. AIF-1 expression modulates proliferation of human vascular smooth muscle cells by autocrine expression of G-CSF. Arterioscler Thromb Vasc Biol 2004; 24:1217-1222.

5 Chen X, Vodanovic-Jankovic S, Johnson B, Keller M. Absence of regulatory T-cell control of TH1 and TH17 cells is responsible for the autoimmune-mediated pathology in chronic graft-versus-host disease. Blood. 2007; 110:3804–3813.

6 Grimm PC, McKenna R, Nickerson P, Russell ME, Gough J. Clinical rejection is distinguished from subclinical rejection by increased infiltration by a population of activated macrophages. J Am Soc Nephrol1999; 10:1582–1589.

7 Schluesener H, Seid K, Deininger M, Schwab J. Transient *in vivo* activation of rat brain macrophages/microglial cells and astrocytes by immune-stimulatory multiple CpG oligonucleotides. J Neuroimmunol 2001; 113:89–94.

8 Kuschel R, Deininger MH, Meyermann R, Bornemann A. Allograft inflammatory factor-1 is expressed by macrophages in injured skeletal muscle and abrogates proliferation and differentiation of satellite cells. J Neuropathol Exp Neurol 2000; 59:323–332.

9 American Diabetes Association. Standards of medical care in diabetes – 2014. Diabetes Care 2014; 37:S15.

10 National Kidney Foundation. National Kidney Foundation K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification and stratification. Am J Kidney Dis 2002; 39:S1.

11 National Institutes of Health (NIH), National Heart, Lung, and Blood Institute (NHLBI). *The practical guide: identification, evaluation, and treatment of overweight and obesity in adults [NIH publication no. 00-4084]*. Bethesda: National Institutes of Health; 2000.

12 Bek T. Experimental approaches to diabetic retinopathy. In: Hammes HP, Porta M, editors. *Clinical presentations and pathological correlates of retinopathy*. *Front diabetes*. Basel: Karger; 2010; 1–19.

13 Levey A, Stevens L. Estimating GFR using the CKD epidemiology collaboration (CKD-EPI) creatinine equation: more accurate GFR estimates, lower CKD prevalence estimates, & better risk predictions. Am J Kidney Dis2010; 55:622-627.

14 Weykamp C, John WG, Mosca A, Hoshino T, Little R. The IFCC reference measurement system for HbA1c: a 6 year progress report. Clin Chem 2008; 54:240-248.

15 Yang ZF, Ho DW, Lau CK, Lam CT, Lum CT. Allograft inflammatory factor-1 (AIF-1) is crucial for the survival and pro-inflammatory activity of macrophages. Int Immunol 2005; 17:1391–1397

16 American Diabetes Association. Standards of medical care in diabetes – 2008. Diabetes Care 2008; 31:S12-S54.

17 Park Y-H, Shin JA, Han J-H, Park Y-M, Yim HW. The association between chronic kidney disease and diabetic retinopathy: the Korea National Health and Nutrition Examination Survey 2008–2010. PLoS One 2015; 10:e0125338.

18 Furtner M, Kiechl S, Mair A, Seppi K, Weger S. Urinary albumin excretion is independently associated with carotid and femoral artery atherosclerosis in the general population. Eur Heart J 2005; 26:279-287.

19 Tesch GH. Role of macrophages in complications of type 2 diabetes. Clin Exp Pharmacol Physiol 2007; 34:1016-1019.

20 Tesch GH. Macrophages and diabetic nephropathy. Semin Nephrol2010; 30:290-301.

21 Harris C. Diabetes and the kidney [chapter 124]. In: Goldman L, Schafer AI, editors. *Goldman–Cecil medicine*. 25th ed. Philadelphia: Elsevier; 2015. 804-806.

22 Fukui M, Tanaka M, Asano M, Yamazaki M, Imai S. Serum allograft inflammatory factor-1 is a novel marker for diabetic nephropathy. Diabetes Res Clin Pract 2012; 97:146–150.

23 Valmadrid CT, Klein R, Moss SE, Klein BE. The risk of cardiovascular disease mortality associated with microalbuminuria and gross proteinuria in persons with older-onset diabetes mellitus. Arch Intern Med 2000; 160:1093–1100.

24 Chow FY, Nikolic-Paterson DJ, Ozols E, Atkins RC, Tesch GH. Intercellular adhesion molecule-1 deficiency is protective against nephropathy in type 2 diabetic db/db mice. J Am Soc Nephrol 2005; 16: 1711-1722.

25 Arner P. Human fat cell lipolysis: biochemistry, regulation and clinical role. Best Pract Res Clin Endocrinol Metab 2005, 19:471–482.

26 Weisberg SP, McCann D, Desai M, Leibel RL. Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest 2003, 112:1796–1808.

27 Dahlman I, Elsen M, Tennagels N, Korn M. Functional annotation of the human fat cell secretome. Arch Physiol Biochem 2012, 118:84 –91.

28 Thorleifsson G, Walters GB, Gudbjartsson DF, Thorsteinsdottir U. Genome-wide association yields new sequence variants at seven loci that associate with measures of obesity. Nat Genet 2009, 41:18–24.

29 Casimiro I. *Role of allograft inflammatory factor-1 (AIF-1) in macrophage activity, atherosclerosis, and obesity*. New York: Yeshiva University; 2010.

30 Lorente-Cebrián, S, Decaunes, P, Dungner E, Bouloumie A, Arner P, Dahlman I. Allograft inflammatory factor 1 (AIF-1) is a new human adipokine involved in adipose inflammation in obese women. BMC Endocr Disord 2013; 13:54.

31 American Diabetes Association. Microvascular complications and foot care. Sec. 9. In Standards of Medical Care in Diabetes 2015. Diabetes Care 2015; 38:S58–S66.

32 Boyle JJ, Weissberg PL Bennett MR. Tumor necrosis factor α promotes macrophage-induced vascular smooth muscle cell apoptosis by direct and autocrine mechanisms. Arterioscler Thromb Vasc Biol 2003; 23:1553–1558.

33 Kuwabara T, Mori K, Mukoyama M, Yokoi H. Macrophage-mediated gluco-lipotoxicity via myeloid-related protein 8/toll-like receptor 4 signaling in diabetic nephropathy. Clin Exp Nephrol. 2014; 18:584–592.

34 Nguyen G. Renin/prorenin receptors. Kidney Int 2006; 69:1503-1506.

35 Kanetsuna Y, Takahashi K, Nagata M, Gannon MA. Deficiency of endothelial nitric-oxide synthase confers susceptibility to diabetic nephropathy in nephropathy-resistant inbred mice. Am J Pathol 2007:170:1473-1484.

36 Powers AC; Diabetes mellitus: diagnosis, classification, and pathophysiology [Chapter 417] In: Longo DL, Fauci AS, Kasper DL, Hauser SL, Jameson JL, Loscalzo J: *Harrison's principles of internal medicine*, 19th ed. New York: McGraw–Hill; 2015. 2399–2406.

37 Kuwabara T, Mori K, Mukoyama M, Kasahara M. Exacerbation of diabetic nephropathy by hyperlipidaemia is mediated by toll-like receptor 4 in mice. Diabetologia 2012; 55:2256-2266.

38 Colhoun HM, Betteridge DJ, Durrington PN, Hitman GA, Neil HACARDS Investigators. Effects of atorvastatin on kidney outcomes and cardiovascular disease in patients with diabetes: an analysis from the Collaborative Atorvastatin Diabetes Study (CARDS). Am J Kidney Dis2009; 54:810-819.

39 Baigent C, Landray MJ, Reith C, Emberson J SHARP Investigators. The effects of lowering LDL cholesterol with simvastatin plus ezetimibe in patients with chronic kidney disease (Study of Heart and Renal Protection): a randomized placebo-controlled trial. Lancet. 2011; 377:2181-2192.

40 Wu M, Chen Y, Wilson K, Chirindel A. Intra-retinal leakage and oxidation of LDL in diabetic retinopathy. Invest Ophthalmol Vis Sc. 2008; 49:2679–2685.

41 Cherney DZ, Sochett EB, Miller JA. Gender differences in renal responses to hyperglycemia and angiotensin-converting enzyme inhibition in diabetes. Kidney int. 2005; 68:1722-1728.

42 Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. N Engl J Med 2005; 352: 1685–1695.

43 Autieri MV, Carbone CM. Overexpression of allograft inflammatory factor-1 promotes proliferation of vascular smooth muscle cells by cell cycle deregulation. Arterioscler Thromb Vasc Biol 2001; 9:1421–1426.

44 Tian Y, Jain S, Kelemen SE, Autieri MV. AIF-1 expression regulates endothelial cell activation, signal transduction, and vasculogenesis. Am J Physiol Cell Physiol 2009; 296:256 –266.

45 Mishima T, Iwabuchi K, Fujii S, Tanaka SY. Allograft inflammatory factor-1 augments macrophage phagocytotic activity and accelerates the progression of atherosclerosis in ApoE$^{-/-}$ mice. Int J Mol Med2008; 21:181-187.

46 Wang Z, Wei M, Wang M, Chen L, Liu H. Inhibition of macrophage migration inhibitory factor reduces diabetic nephropathy in type II diabetes mice. Inflammation. 2014; 37:2020-2029.