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Serum Osteoinductive Factor as potential Marker of Nephropathy in Type 1 Diabetes Mellitus

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Abstract: Background: The association among serum osteoinductive factor (OIF) and diabetic nephropathy (DN) and its possible usage as a diagnostic marker for DN should be investigated. Subjects and methods: This study included a total of 60 type 1 diabetic patients divided to normoalbuminuria, microalbuminuria and macroalbuminuria groups , each group contained 20 patients in addition to 20 healthy control subjects. ELISA has examined serum OIF levels and checked other clinical biochemical parameters on the basis of standard methods. Results: The concentration of OIF in microalbuminuric and macroalbuminuric patients was substantially higher than in normoalbuminuric patients and control groups. The study showed significant positive correlation between serum OIF, DM duration, Creatinine and UACR but significantly negative correlated with eGFR and onset of diabetes. Conclusion: Our results indicated that OIF was demonstrated in early stages of DN in T1DM even before appearance of microalbuminuria and increased with progression of diabetic nephropathy. So OIF can be used as a biomarker for detection of DN in T1DM in microalbuminuric patients.

Keywords: Osteoinductive factor; Diabetes mellitus; Diabetic nephropathy

Abbreviations: OIF =Osteoinductive factor**, UACR** = urinary

INTRODUCTION

Type 1diabetes Mellitus (T1DM) is a chronic metabolic disease of carbohydrate, fat, and protein occurring in childhood as a consequence of autoimmune destruction of pancreatic beta cells (1). Diabetic nephropathy (DN) is considered to be a major trigger of end-stage

renal diseases in diabetic patients as a microvascular complication in both types of diabetes mellitus (2). The characteristics of diabetic nephropathy include renal cell hypertrophy, thickening of the glomerular basement membrane, accumulation of extracellular matrix proteins and mesangial cell expansion (3). Osteoinductive factor(OIF) is a secretory protein that was primarily known to stimulate ectopic bone formation(4). Osteoinductive factor is incorporated into the normal vascular matrix and has significant roles in lipid metabolism and carbohydrate metabolism(5). Microalbuminuria is an early marker of diabetic nephropathy, but some diabetic nephropathy patients with early glomerular influence may present with a normal range of albumin in the urine. so, we need more sensitive markers of early detection of diabetic nephropathy(6)

Yang et al., (2007) reported that microalbuminuria is a standard marker of DN. Sadly, excretion, sports, urinary tract infection; high blood pressure, heart failure, and fever have easily interfered with this marker. The epidemiological investigation of diabetic patients showed that 44.3% of patients who got diabetic nephropathy were normoalbuminuric. So, microalbuminuria cannot completely demonstrate the risk of DN (7).

Early detection of diabetic nephropathy is important for management and end-stage kidney disease prevention. Persistent albuminuria and elevated serum creatinine with gradual decrease in eGFR characterize diabetic kidney disease. DN staging depends on different markers, some of which are successful only at the late stages of the disease, like eGFR. Other indicators such as albuminuria are a dynamic, fluctuating condition (**8).**

Therefore, OIF has been recently suggested to have a role in the glomerular pathology associated with diabetic nephropathy, moreover; it was studied as a novel biomarker of early diabetic nephropathy (**14**).

MATERIALS AND METHODS

A case control study was performed at the Department of Internal Medicine, Zagazig University Hospitals from September 2018 to august 2019 on type 1 diabetic patients. Ethical consideration: Written consent was obtained from every patient after explanation of the research. Medical research and ethics committee of Zagazig University approved the study. The study was performed in compliance with the 2013 World Medical Association Code of Ethics (Helsinki Declaration) for studies including humans.

Subjects : This study included 60 type 1 diabetic patients classified to normoalbuminuria , microalbuminuria and macroalbuminuria groups ,20 cases for each group in addition to 20 healthy individuals as control group. All patients underwent a medical and clinical history taking and a complete clinical assessment for select patients with diabetic nephropathy. Established type 1diabetes mellitus (diagnosed according to WHO criteria (i.e., fasting blood glucose (FBG) ≥ 126 mg/dL, postprandial blood glucose ≥ 200 mg/dL, DM symptoms with randomised blood glucose ≥ 200 mg/dL, or A1C ≥ 6.5%), age more than 15 years, under insulin therapy were included in the study. Exclusion criteria were: (1)Acute metabolic disorders involving ketoacidosis, hyperglycemia, hyperosmolar status.(2)Acute severe infection. (3)Patients on hemodialysis.(4) Patients with a bone fracture within the previous 3 months.(5) Autoimmune diseases. (6)Malignancies.(7) Coronary heart disease.(8) Liver diseases. (9)Acute cerebrovascular accidents. (10)Patients with abnormal renal ultrasound beyond diabetic nephropathy.

Sampling and laboratory investigation:

Kidney function tests, fasting blood sugar (FBS), HbA1c, serum total cholesterol, serum triglycerides, serum HDL, serum LDL, UACR. 3 ml of blood collected with tube then centrifuged at 3,000g for 10 minutes, serum samples separated into Opendorph and kept frozen at -80C until tested, Urine samples were withdrawn for albumin, Urine samples were

withdrawn for albumin and calculation of urine albumin creatinine ratio (UACR) and estimated glomerular filtration rate (eGFR).
Serum OIF (was calculated according to the manufacturer's protocols using an enzyme-linked immunosorbent assay (ELISA) kit). All routine investigations were done and microalbuminuria was done by Immunoturbidometric assay. Chronic Kidney Disease Epidemiology Collaboration Equation, has determined the glomerular filtration rate **(10)**.

Statistical analysis
The data is represented as the mean ± standard deviation value. To compare means among groups with normally distributed data, one-way analysis of variance (ANOVA) has been applied. The median ± interquartile range is viewed as data with a skewed distribution. A 1-way ANOVA was used to analyze skewed data with a normal distribution following logarithmic transformation. A nonparametric test was used to analyze skewed data. The ratio and percentage of the chi-squared test used to compare groups were the enumeration data expressed. Pearson correlation analysis analyzed associations among the serum level of OIF with UAER and eGFR. Using multivariate logistic regression, the predictive value of OIF for the risk of early stage DN was evaluated. To determine the cut-off value of OIF for forecasting early DN, receiver operating characteristic (ROC) analysis was performed. All analyses have been conducted using version 17.0 of the Statistical Package for Social Sciences (SPSS, Chicago, IL). Variations to $P < .05$ are found to be significant in all statistical tests.

RESULTS AND DISCUSSION

Demographic information were collected; the enrolled number of the study was 80 participants, among them 36 males and 44 females.
Table (1) : The mean ages for groups I, II, III and IV were 19.54±1.85, 20.95±2.09, 21.3±2.19 and 21.4±2.3, without substantial difference among the groups, respectively, also there was no significant difference among studied subjects as regards to BMI, sex or smoking habit according to study design. DM duration in macroalbuminuric and microalbuminuric patients was substantially higher than in normoalbuminuric subjects. There was no significant difference or association regarding HTN but diabetic retinopathy was significantly higher among macroalbuminuric patients.

			Control	Normoalbuminuria	Microalbuminuria	Macroalbuminuria	F/ X^2	P
Age (years)			**19.54 ±1.85**	**20.95±2.09**	**21.3±2.19**	**21.4±2.3**	**1.458**	**0.141**
Body Mass Index (kg/m2)			**23.39 ±2.27**	**25.39±3.39**	**25.95±2.58**	**26.16±6.2**	**0.354**	**0.789**
Diabetes duration (years)			**----------**	**3.67±1.21#**	**7.9±2.46**	**11.85±2.46**	**97.970**	**0.00**
Sex	**Female**	**N**	**10**	**13**	**10**	**11**		
		%	**50.0 %**	**65.0%**	**50.0%**	**55.0%**		
	Male	**N**	**10**	**7**	**10**	**9**	**2.09**	**0.62**

		%	50.0 %	35.0%	50.0%	45.0%		
Smoking	No	N	18	19	17	16		
		%	90.0 %	95.0%	85.0%	80.0%		
	Smoker	N	2	1	3	4	2.28	0.51
		%	10.0 %	5.0%	15.0%	20.0%		
Hypertension	-VE	N	20	20	19	17		
		%	100.0 %	100.0%	95.0%	85.0%		
	+VE	N	0	0	1	3	4.21	0.13
		%	0.0%	0.0%	5.0%	15.0%		
Retinopathy	-VE	N	20	20	19	15		
		%	100.0 %	100.0%	95.0%	75.0%		
	+VE	N	0	0	1	5	8.25	0.02
		%	0.0%	0.0%	5.0%	25.0%		
Total		N	20	20	20	20		
		%	100.0 %	100.0%	100.0%	100.0%		

Table (2): Vital data distribution between studied groups

	Control	Normo albuminuria	Micro albuminuria	Macro albuminuria	F	P
SBP (mm Hg)	112.2±7.24	116.5±8.12	124.5±9.44	126.5±8.75	8.874	0.002
DBP (mm Hg)	73.8±9.9	76.0±8.2	83.0±7.32	83.0±4.71	8.254	0.002

Table (3): Lipid profile distribution allover studied groups

	Control	Normo albuminuria	Micro albuminuria	Macro albuminuria	F	P
Triglyceride(mg/dL)	75.69±8.36	100.7±33.74	110.3±35.6	192.85±68.1	19.987	0.00
Cholesterol(mg/dL)	102.85±11.8	160.35±30.7	170.85±27.1	190.85±47.6	3.874	0.032
HDL(mg/dL)	47.92±4.92	40.05±13.45	42.35±10.2	37.7±9.94	3.587	0.045

LDL (mg/dL)	**68.05±9.91**	**68.95±20.24**	**85.3±28.23**	**79.05±16.84**	**2.860**	**0.073**

Table (4): Kidney function distribution among studied groups

	Control	**Normo albuminuria**	**Micro albuminuria**	**Macro albuminuria**	**F/ kruskal Walis**	**P**
Creatinine (mg/dL)	**0.76±0.104**	**0.76±0.15**	**0.92±0.23**	**2.42±0.12**	**12.654**	**0.00**
Urea (mg/dL)	**29.25±2.63**	**31.15±5.65**	**32.4±7.1**	**9.85±5.51**	**1.547**	**0.231**
UACR	**8.10±2.26**	**8.32±4.6**	**79.54±39.8**	**625.2±115.5**	**K 354.85**	**0.00**
e GFR (CKD-EPI)	**120.55±19.68**	**116.65±19.68**	**110.0±19.9**	**75.0±10.9**	**19.974**	**0.00**

Table (5) : OIF was positively correlated with DM duration, Creatinine and UACR but a significant negatively correlated with eGFR

		OIF
BMI	**r**	**.044**
	p	**.736**
DM duration	**r**	**.508**
	p	**.000**
SBP	**r**	**.452**
	p	**.000**
DBP	**r**	**.425**
	p	**.000**
FBS	**r**	**.132**
	p	**.315**
A1C	**r**	**.185**
	p	**.157**
Triglyceride	**r**	**.109**
	p	**.406**
Cholesterol	**r**	**.178**
	p	**.173**
HDL	**r**	**.059**
	p	**.656**
LDL	**r**	**.075**
	p	**.568**
Creatinine	**r**	**.367**
	p	**.004**
UREA	**r**	**.219**
	p	**.058**
UACR	**r**	**.395**
	p	**.002**
e GFR	**r**	**-.377**
	p	**.003**

Table (6): OIF concentration was substantially higher in microalbuminuric patients and macroalbuminuric subjects than in normoalbuminuric and control groups.

	Control	Normo albuminuria	Micro albuminuria	Macro albuminuria	F	P
OIF (pg/dl)	276.99±15.58	285.7±25.68	328.85±34.2	341.55±27.54	24.697	0.00

Table (7): Significant area under curve with significant cutoff (>314.5) with sensitivity 80% and specificity 75.0%

Area	Cutoff	P	95% Confidence Interval		Sensitivity	Specificity
			Lower Bound	Upper Bound		
0.893	>314.5	0.00	0.815	0.972	80.0%	75.0%

DISCUSSION

Diabetic nephropathy is a chronic complication of long standing and poorly controlled diabetes mellitus, Occurs in 20% to 40% of patients with diabetes (11). Type 1 diabetes morphological lesions (T1DM) primarily impact glomeruli, with basement membrane thickening and mesangial expansion, often affecting podocytes, renal tubules, interstitium and arterioles, particularly at later stages of disease (12). In the development of angiogenesis and atherosclerosis, Serum OIF is involved. Vascular endothelial damage was shown to be caused by vulnerable hemorrhagic carotid and coronary atherosclerotic plaques and can have prognostic significance in coronary artery disease patients as well (13).OIF can be a possible biomarker for the diagnosis and assessment of DN initiation and development between DM patients (14).

In order to further explore the association among serum OIF and DN, to examine the potential mechanism connecting OIF and DN, and to evaluate the value of serum OIF in the early diagnosis and surveillance of DN, we performed this case control study. We assumed that serum OIF has been involved in DN pathogenesis and development and could be utilized as a DN diagnostic marker in patients with type 1 DM even before microalbuminuria.

In our study according to study design, there was no substantial difference in age, BMI, sex or smoking habits among the control group and patients with diabetes and This result is similar to result obtained by Wang et al.(14) El-Beblawy et al.(15) and Wei et al (9).

Our results reviewed that DM duration was significantly higher in macroalbuminuric and microalbuminuric patients than in normoalbuminuric subjects and this is in line with the studies of (Rodrigues et al.(17), (El-Beblawy et al(15) who reported that The prevalence of DN associated with T1DM increased with a longer duration of diabetes and usually occurs after 5 years of diabetic duration . Despite that there was no significant difference in history of hypertension in our study patients, measurements of systolic blood pressure and Diastolic blood pressure exhibited significantly higher level in microalbuminuric and macroalbuminuric patients than normoalbuminuric subjects. Variable proposed mechanisms can explain the pathogenic role of diabetes mellitus in development of hypertension involving systemic vascular inflammation , endothelial dysfunction, alterations in atrial natriuretic peptide, and renin-angiotensin system (Stehouwer et al .(17) , Darcan et al.(18) and (Wang et al.,(14) who reported that hypertension plays a significant role in the development of persistent microalbuminuria, promoting the hypothesis that glomerular hypertension is essential to the initiation and progress of diabetic kidney disease. Hypertension has harmful effects within the glomerulus by inducing impaired autoregulation of the glomerular

microcirculation. This involves both afferent and efferent arteriole vasodilatation, which has a more vigorous impact on the afferent arteriole, contributing to a rise in intraglomerular capillary pressure.(Hostetter, et al(19) and Liu, (20).

Clinically microalbuminuria is the earliest manifestation of diabetic nephropathy (21)Concerning UACR Macroalbuminuric, patients were significantly associated with the highest levels and with the lowest GFR. And this is agree with (Wang et al (14).

Yang et al., (2007) reported that microalbuminuria is a standard marker for DN.but, this marker easily affected by excretion, sports, urinary tract infection, high blood pressure, heart failure, and fever. The epidemiological investigation of diabetic patients showed that 44.3% of patients who got diabetic kidney diseases were normoalbuminuric(7). Microalbuminuric, therefore, may still not fully explain whether patients may or may not be at risk for DN.

For better clinical care and to avoid approaching the end stage of renal disease, early detection of diabetic kidney disease is important. Persistent albuminuria and elevated serum creatinine with gradual decrease in eGFR characterize diabetic nephropathy. The DN stage is controlled by multiple indicators, several of which are only successful at late stages of the disease, like eGFR. The dynamic, fluctuating state of other indicators such as albuminuria (8) .

The existence of albuminuria is generally recognized as an indicator of progressed renal structural alterations in the kidney, representing the established nephropathy status. (22)

Our results showed marked deterioration of eGFR in macroalbuminuric patients correlating with diabetic nephropathy progression.

This finding was in agreement in the study published by (Wang et al., 2015) who reported a rise in OIF serum concentrations in individuals with DN and OIF became a sensitive indicator for early microalbuminuria (14). In comparison with healthy and T2DM individuals, serum OIF levels were shown to be substantially increased in DN individuals. Correlation studies have shown that OIF was associated with creatinine positively and eGFR negatively. These findings indicated that OIF levels in individuals with DN were closely correlated with renal function deterioration. We found that serum OIF had high sensitivity and specificity for the prediction of microalbuminuria by carrying out the ROC plots, so the findings showed the potential role of serum OIF levels for the initiation and progression of DN in individuals with DM.

We reported a substantial increase in serum OIF levels in DN patients relative to people with T1D in our study.

In addition, clinical diabetic nephropathy indicators such as UACR and serum creatinine have been positively associated with serum OIF, whereas eGFR reflected glomerular filtration rate has been negatively associated with serum OIF.

In our study population, OIF start to rise early in diabetic patients with diabetic duration less than 5 years with normoalbuminuria and progressive rise with progression of nephropathy, suggesting serum OIF as an early marker of nephropathy.

Therefore, measurement of serum osteoinductive factor provides a suitable biomarker for early detection and monitoring of diagnosis of diabetic nephropathy in type 1 DM.

CONCLUSION

OIF was demonstrated in early stages of DN inT1DM even before appearance of microalbuminuria and increased with progression of diabetic nephropathy.

So OIF can be used as a biomarker for early detection of DN in T1DM in microalbuminuric patients.

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Conflicts of Interest/ Competing Interests
No conflict of interest

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