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Study the Levels of Serum Myo-Inositol Oxygenase (MIOX) in Type2 Diabetic Patients with Albuminuria

Marwa Abbas Abd (B.S.C) [1]. Hassan H. Al-Saeed (PhD) [2]. Arif Sami Malik (Prof) [3]

1. Medical Analysis Tech**.** Al-Rasheed University College.
2. Department of Chemistry and Biochemistry, College of Medicine, Al-Nahrain University.
3. Consultant Nephrologyst, Department of internal medicine, College of medicine/ Al-Nahrain University.

Abstract Diabetic nephropathy (DN) is one of the most common diabetic microvascular complication, which occurs in 20% to 40% of all patients with type 2 diabetes mellitus, is a metabolic disorder with high morbidity and mortality. The classical description of DN is a albumin in urine grows slowly and gradually. Myo-Inositol Oxygenase (MIOX) is regarded to be the most powerful organizer of Myo-inositol (MI) MI is mainly metabolized into D-glucuronic acid in the renal.

Objective To evaluate the role of early detection power of serum biomarkers irisin and MIOX for the diagnosis of type 2 diabetic patients with albuminuria.

Subjects and methods: a research implement within 160 entrant (illness and standard). The patient groups separated to: (40 T2DM.W.A), (40 T2DM.Mi) and (40 T2DM.Ma), (40 controls).This is a case control research. MIOX grads was measure by ELIZA, around 7ml of blood specimen were collected from patients and control. The excluded criteria of the study were patients Type1diabetes Mellitus, Recent type2 D.M. associated diseases with (heart failure, autoimmune disorders), No laboratory sign of hematuria or UTI, History of prolonged use of nephrotoxic drugs, Nephrotic range of proteinuria, End stage renal disease (ESRD), Patient's below 40 years old or older than 70 years old.

Result The patient groups with Type2 Diabetic Patients with Albuminuria present an eloquent rising in Serum MIOX grads in rapprochement to standard group ($p < 0.001$).

Conclusion A rise grade of Serum MIOX was diagnosed clinically who had Type2 Diabetic Patients with Albuminuria. Assay may be used as promoting biochemical markers for the early diagnosis and predicting of type 2diabetic patients with albuminuria

Keywords serum MIOX, Biomarker, Type 2 diabetic with albuminuria.

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Background:

Diabetic nephropathy is marked by a steady rise in albuminuria, accompanied by a decline in (eGFR) afterward in the disorder, which may contribute to end stage renal disease (ESRD) (See Figures 1 and 2) (1) Diabetic nephropathy is a long-term symptom of both type 1 and type 2 diabetes mellitus (2). Diabetic kidney failure, also known as diabetic nephropathy, is by far the most prevalent risk factor for diabetes in both industrialized and developing economies, advancing to end stage renal disease (ESRD) and mortality. It's worth noting that the raised risk of mortality on any reason in diabetic patients is specifically related to the prevalence of kidney failure (3). Long-term diabetes, irregular glycemic, serum cholesterol, and, overweight, tobacco are also important determinants. Much of these lifestyle factors may be adjusted (1). The occurrence of vascular complications is intimately associated to the elevated hyperglycemia condition of diabetic. In adults, though, the proof of a strong causal association among insulin resistance and kidney problems is poorer than in animal studies. Diabetic nephropathy is marked by a steady spike in albumin, dyslipidemia, and a reduction in glomerular activity, which both eventually lead to kidney failure. Diabetes patients are likely to inherit that disorder as a result of related conditions such as dyslipidemia and hypertension. The mortality rates are high, consequent to cardiac disease (4). Myo-inositol oxygenase (MIOX), is regarded to be the most powerful organizer of Myo-inositol (MI) MI is mainly metabolized into D-glucuronic acid in the renal (6). Previously, an laboratory cloned a renal-specific oxido-reductase gene from a human cDNA library and initially designated it as RSOR (7).Now, it has been uniformly named as myo-inositol oxygenase (MIOX) (5).the previous determined that MIOX expression is confined to renal proximal tubular cells and found that it is upregulated in the kidney of diabetic mouse. Current researches have shown that MIOX not only regulates MI metabolism but also participates in the pathogenesis of D.N. tubular injury through non-MI metabolic pathways, such as by stimulating the release of TGF-β (8), disrupting the redox status (9) and regulating mitochondrial dynamics (10). Interestingly, Gaut et al. found that an increase in serum MIOX preceded the elevation of serum creatinine in patients with acute kidney injury (AKI), which was closely associated with the degree of tubular damage (11), indicating that serum MIOX can be used as an AKI biomarker. Despite the evident importance of

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MIOX in streptozotocin-induced diabetic mice tubular injury, little is known about its role and diagnostic value in D.N. patients (12).

Subjects and Study Protocol:

1. The study was performing during the period from January 2020 to May 2020 and 160 peoples were participated in it. **Inclusion criteria** Patients age between 40-70 years old with and without type 2 diabetes albuminuria who presented to who Al-Imamin AL-kadhimeen City hospital and al-karama teaching hospital Depending on the diagnosis. **Exclusion criteria** Type1diabetes Mellitus, Recent type2 D.M. associated diseases with (heart failure, autoimmune disorders),No laboratory sign of hematuria or UTI, History of prolonged use of nephrotoxic drugs, Nephrotic range of proteinuria, end stage renal disease (ESRD) ,Patient's below 40 years old or older than 70 years old

Sample collection and methods:

About seven milliliters of blood were collected from vein puncture of the overnight fasting patients and controls .The blood samples were divided into two parts Part one: about two milliliters were collected into EDTA containing tubes and sent to the laboratory for measuring glycated HbAlc Part two: the blood samples were left for 20 minutes at room temperature. After coagulation, sera were separated by centrifugation at 2000 xg for 10 min. Immediate measurements of serum glucose, serum urea and serum creatinine, lipid profile and the rest were stored at -20 until assayed for myo-inositol oxygenase (MIOX) will be measured using by(ELISA) kits

Results: A total sample of (180) participants, (120) patients consist from (40) (T2DM.W.A), (40) (T2DM.Mi) and (40) (T2DM.Ma) and 40 control. General characteristics of all studied groups (T2DM.W.A, T2DM.Mi, T2DM.Ma and control) which include age, BMI are demonstrated in table (1). The choice of the age that was taken in this study was range from (40-70) years old, the result in table (1) show there is significant difference in age between studied groups ($P< 0.0001$). In addition, the result showed significant variation among groups regarding BMI between studied groups ($p < 0.0001$).

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Table 1: Features of a Research Teams in Particular:

Statistic	No.	Mean ± SD	Median ± SEM	Skewness	Kurtosis	P
Age						
Control	60	51.317±5.75	51.5 ±0.742	-0.01	-1.16	< **0.0001**
T2DM.W.A	40	50.2 ± 6.62	50±1.047	0.26	-0.74	
T2DM.Mi	40	56.95±7.25	57±1.146	-0.56	-0.26	
T2DM.Ma	40	58.77 ± 9.6	60.5±1.521	-0.90	-0.42	
BMI						
Control	60	25.19 ± 2.68	25.38 ± 0.35	-0.29	-0.72	< **0.0001**
T2DM.W.A	40	30.73 ± 2.77	31.22 ± 0.44	-1.19	1.16	
T2DM.Mi	40	27.91 ± 2.69	27.00 ± 0.43	1.04	0.05	
T2DM.Ma	40	26.88 ± 4.22	25.75 ± 0.67	0.05	-1.50	

The mean and standard deviation (Mean ± SD), Skewness, Kurtosis, Median ± SEM and p value for all biochemical tests measured as shown in the table (2) below:

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Table 2: Biochemical Tests in the Study Groups

Groups	*N*	Mean ± SD	Median ± SEM	Skewness	Kurtosis	*p-value*
MIOX						
Control	60	16.20 ± 0.658	16.49 ± 5.09	1.544	5.28	**< 0.001***
T2DM.W.A	40	41.65 ± 2.016	44.33 ± 12.75	0.988	2.73	
T2DM.Mi	40	64.70 ± 1.918	59.58 ± 12.13	-2.399	5.99	
T2DM.Ma	40	80.10 ± 3.34	69.89 ± 21.12	-1.905	2.16	
TC						
Control	60	181.24 ± 18.91	188.00 ± 2.44	-1.13	0.21	**< 0.0001**
T2DM.W.A	40	218.44 ± 50.11	238.25 ± 7.92	-0.24	-1.44	
T2DM.Mi	40	229.48 ± 59.55	254.60 ± 9.42	-0.22	-1.55	
T2DM.Ma	40	236.71 ± 66.02	264.10 ± 10.44	-0.20	-1.60	
TG						
Control	60	144.28 ± 16.21	149.50 ± 2.09	-2.70	6.17	**0.001**
T2DM.W.A	40	179.70 ± 89.37	156.55 ± 14.13	0.38	-1.28	
T2DM.Mi	40	200.65 ± 100.96	19 .50 ± 1 .96	0.04	-1.65	
T2DM.Ma	40	203.83 ± 102.61	193.50 ± 16.22	-0.02	-1.67	
HDL						
Control	60	44.03 ± 3.15	43.90 ± 0.41	0.31	0.54	**< 0.0001**
T2DM.W.A	40	43.90 ± 3.13	43.60 ± 0.49	0.20	-1.15	
T2DM.Mi	40	40.07 ± 6.06	40.50 ± 0.96	-0.56	-0.35	
T2DM.Ma	40	39.54 ± 9.19	37.65 ± 1.45	0.25	-0.94	
LDL						
Control	60	104.41 ± 22.53	104.65 ± 2.91	-0.17	-1.48	**< 0.0001**
T2DM.W.A	40	132.29 ± 44.52	135.80 ± 7.04	0.04	-1.12	
T2DM.Mi	40	152.08 ± 54.48	165.50 ± 8.61	-0.24	-1.24	
T2DM.Ma	40	158.07 ± 59.81	171.85 ± 9.46	-0.10	-1.04	
VLDL						
Control	60	28.88 ± 3.25	29.95 ± 0.42	-2.72	6.23	**0.001**
T2DM.W.A	40	36.23 ± 18.35	31.00 ± 2.90	0.37	-1.35	
T2DM.Mi	40	40.46 ± 20.59	38.50 ± 3.26	0.01	-1.69	
T2DM.Ma	40	41.18 ± 20.80	38.70 ± 3.29	-0.05	-1.71	
B.Urea						
Control	60	41.14 ± 1.00	40.80 ± 0.13	2.35	8.61	**< 0.0001**
T2DM.W.A	40	43.02 ± 1.77	43.20 ± 0.28	-1.79	6.14	
T2DM.Mi	40	74.24 ± 7.69	74.10 ± 1.22	-0.10	-1.26	
T2DM.Ma	40	82.20 ± 5.85	81.05 ± 0.93	0.02	-1.00	
Creatinine						

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Control	60	0.56 ± 0.08	0.56 ± 0.01	0.10	-0.73	**< 0.0001**
T2DM.W.A	40	0.63 ± 0.10	0.60 ± 0.02	-0.19	-0.52	
T2DM.Mi	40	1.58 ± 0.30	1.60 ± 0.05	-0.70	2.71	
T2DM.Ma	40	3.09 ± 0.62	3.10 ± 0.10	-1.00	2.07	
GFR						
Control	60	117.03 ± 5.79	117.00 ± 0.75	0.08	-0.68	**< 0.0001**
T2DM.W.A	40	111.65 ± 5.65	110.50 ± 0.89	0.79	0.64	
T2DM.Mi	40	42.87 ± 9.45	42.00 ± 1.49	-0.28	0.20	
T2DM.Ma	40	19.20 ± 2.17	19.00 ± 0.34	-0.12	-0.85	
FBS						
Control	60	100.00 ± 5.60	100.00 ± 0.72	-0.35	-0.81	**< 0.0001***
T2DM.W.A	40	196.54 ± 48.98	189.00 ± 7.74	1.95	5.15	
T2DM.Mi	40	223.10 ± 13.77	221.00 ± 2.18	4.65	24.02	
T2DM.Ma	40	237.43 ± 25.36	238.00 ± 4.01	1.18	1.55	
HbA1c						
Control	60	5.74 ± 0.16	5.80 ± 0.02	-1.01	0.91	**< 0.0001**
T2DM.W.A	40	7.96 ± 0.56	8.00 ± 0.09	0.06	-0.31	
T2DM.Mi	40	8.53 ± 1.02	8.40 ± 0.16	0.34	-1.07	
T2DM.Ma	40	8.73 ± 0.57	8.80 ± 0.09	-0.12	-0.92	

Pr > F Analysis of variance.

Serum MIOX was analyzed as a predictive biomarker for the detection of Type 2 diabetic patients with albuminuria using the Receiver Operator Characteristic (ROC) Curve, give a highly significant

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difference for all groups (p value < 0.001). As shown in figure (1)

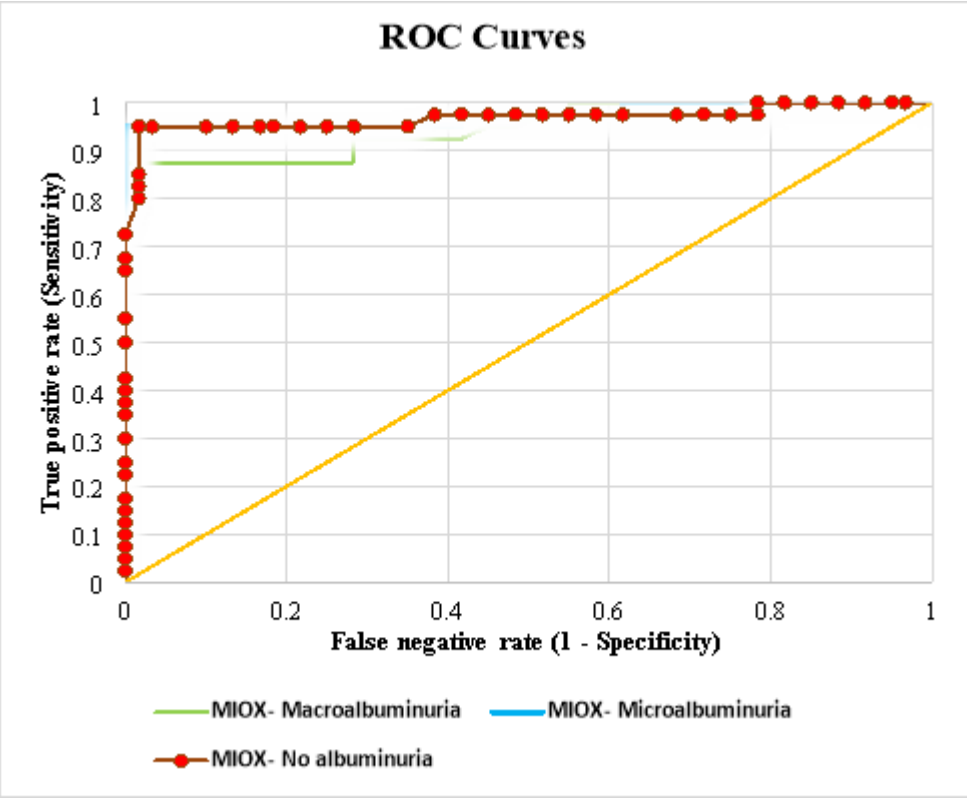

Figure (1) Receiver operator characteristic curves of serum MIOX. For diabetic patient groups as discriminating them from control

Table (3): Sensitivity and Specificity of serum MIOX for T2DM patients versus controls.

	MIOX-T2DM.Ma	MIOX-Microalbu	MIOX- albuminuria N
AUC	0.949	0.974	0.968
Cut-off	39.100	39.100	30.100
Sensitivity	87.5%	95.0%	95%
Specificity	100%	100%	98.3%
Accuracy	95.0%	98.0%	97.0%

Discussion:

Type 2 Diabetes Mellitus is characterized by reduced sensitivity to the action of insulin and an inability to produce sufficient insulin to overcome

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this 'insulin resistance'. Hyperglycemia causes both acute and Long-term problems. Acutely, high glucose and lack of insulin can result in marked symptoms, metabolic decompensation and hospitalization. Chronic hyperglycemia is responsible for diabetes-specific 'microvascular' complications affecting the eyes (retinopathy), kidneys (nephropathy) and feet (neuropathy) (13).The age was match in the three groups of the study. Age are range from 40 to 70 years old of patients with T2diabetes mellitus without albuminuria, T2 diabetes mellitus with microalbuminurai,T2 diabetes mellitus with macroalbuminuria which show significant differences in age between studied groups in patients and control ($p < 0.0001$), as shown in table (1), and this agree with previous study obtained by agree with (14)**.** The result demonstrated that there was highly significant for T2DM.W.A, T2DM.Mi and T2DM.Ma when compared with healthy control ($p<0.0001$), as shown in table (1). These results were agreed with previous (15) and (16) who founds high BMI in T2DM, microvascular and macrovascular complications of type 2 diabetic Mellitus. Also, the present study showed that BMI in T2DM.W.A was more than T2DM.Ma and T2DM.Mi, this maybe contributed to that BMI. In FBS the results showed there were highly significant differences between studied groups ($p< 0.0001$) This study agree with (17) and (18) This may be due to the level of insulin and insulin resistance (IR) increased with the increase in disease duration. The results show highly significant in HbA1c ($p < 0.0001$) as show in table (2) This study agree with (19) who confirmed the hypothesis that HbA1C variability is strongly associated with the development of macroalbuminuria, especially patients under a microalbuminuria state.

The result show there were a significant increase in lipid profile ($p< 0.0001$) as show in table (2) except for HDL levels which deceased this study agree with (Palazhy and Viswanathan, 2017) who found the significant increasing levels of LDL, TG and VLDL-C and significant decrease in level of HDL has the severity of diabetes. Test blood urea highly significant levels showed a significant differences between studied groups ($p < 0.001$), as shown in table (2). The results showed there was elevation in blood urea levels in T2DM.Mi, T2DM.Ma group when compared (T2DM.W.A, control) group, This may be due to overtime high blood sugar level damage millions of nephron, the tiny filtering units in each kidney. This study agree with (20) another review by (21). The results

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showed serum creatinine highly significant ($p < 0.001$) in patient groups with (T2DM.Mi, T2DM.Ma) when compared with (DM.W.A, control). This may be due to longer disease duration; hyperglycemia directly causes mesangial expansion and injury by increasing the mesangial cell glucose concentration. This study agree with ((20) another study by (21). The results study showed the GFR in patient groups were significantly lower ($p < 0.001$) for (T2DM.Ma, T2DM.Mi) group when compared with (T2DM.W.A control) group. Diminished GFR are likely important markers of kidney decline. It is unclear whether these biomarkers reflect independent risk factors for kidney disease progression, early diagnosis, and early initiation of nephroprotective therapy to prevent the progression of DKD toward end-stage renal disease and to improve patients' prognosis. Based on the guidelines issued by the Kidney Disease Outcomes Quality Initiative (KDOQI) in 2007 (22).

ANOVA test was used to calculate the p value of MIOX results. The results showed a significant differences between studied groups ($p< 0.001$), as shown in table (2). The present result demonstrated the level of serum MIOX in (T2DM.Ma) patients group is higher than T2DM. Mi, T2DM.W.A and control group. This may be due to the MIOX responsible for the depletion of MI (Myo-inositol) found in diabetes complications. This study agree with (23) Who found the MIOX was higher in patients groups compared to healthy controls. Another study by (24) who was demonstrate the results of the investigation are based on the idea that the expression MIOX modifies the outcome of tube injury in the case of diabetes by regulating the different signal pathways and the present data testify to this variation and produce an overview of the relevant mechanisms.

Conclusion

The T2DM patients with albuminuria (T2DM.Mi, T2DM.Ma) show increase the level of serum MIOX which may be considered as a predictive markers for early detection of diabetic nephropathy.

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