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Osteoinductive factor as a significant marker for the diagnosis of early diabetic nephropathy

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Abstract
Background: The main factor causing chronic kidney disease, which progressively leads to end-stage renal failure, is diabetic nephropathy (DN). This study aims to shed light on the importance of osteo-inductive factor (OIF) as a sensitive indicator for the early identification of DN
Methods: On 80 participants, this prospective cohort observational research was conducted. A total of 60 of them had diabetes mellitus type 2 based on ADA 2019 guidelines. Four equal groups of patients were created: 20 healthy people made up the group 1 (control group), followed by type 2 diabetes patients with normo-albuminuria in groups 2, micro-albuminuria in groups 3, and macro-albuminuria in groups 4.
Results: A substantial elevation in OIF was existed in group 3 compared to group 1 and group 2, while there were no substantial variations among group 1 and group 2. OIF was directly proportionate to the degree of albuminuria. There was important positive relation between OIF and HbA1c %, fasting blood glucose, 2 hours post prandial blood glucose, serum creatinine, blood urea, urine albumin / creatinine ratio, total cholesterol, TG and Low density lipoproteins, while there was negative correlation between OIF and both of glomerular filtration rate and high-density lipoprotein. OIF is significant predictor for early detection of microalbuminuria (Area under the curve (AUC) was 0.869 and p=0.014) at cut-off 9.0 with 86.7% sensitivity and 95% specificity.
Conclusions: OIF is a sensitive indicator for early microalbuminuria and increases with progression of diabetic nephropathy, also marker of DN and can be considered as a good predictor for early diabetic nephropathy.

Keywords: Diabetic nephropathy, osteoinductive factor, marker

Introduction
A metabolic disorder Known as Diabetes mellitus (DM) is marked by excessively elevated blood glucose levels. It is known as one of the major global causes of disability and mortality [1]Type 1, type 2, diabetes during pregnancy, diabetes with maturity-onset of the young (MODY), diabetes in neonates, and additional causes resulting from endocrine disorders, use of steroids, etc. represent a few of the different types of DM [2].
The body's insufficient use of insulin leads to Type II diabetes mellitus (T2DM), commonly known as adult-onset, non-insulin-dependent. T2DM affects above 95% of patients with diabetes. The main causes of this type of diabetes are being overweight and not exercising enough [3].
Diabetes that is uncontrolled may result in both microvascular and macrovascular consequences, including chronic kidney disease, diabetic neuropathy, retinopathy, and cardiovascular disease, all of which can be fatal [1].
One of the key factors of end-stage renal disorders in people who are diabetic is diabetic nephropathy, which is a microvascular consequence caused by diabetes. Basic characteristics of diabetes-related nephropathy (DN) causing renal fibrosis include mesangial cell growth, glomerular basement layer thickness, matrix of extracellular protein deposits, and renal cell enlargement. A diabetic kidney disease's early recognition is essential for better treatment and to prevent the occurrence of renal failure in its final stages. Microalbuminuria has been recognized as an early marker of diabetic nephropathy; however, growing evidence supports that DN A normal range of albumin in urine may be noticed in people with early glomerular effect.

Hence, there has been a necessary need for more sensitive markers of early diabetic nephropathy [4]

The secretory protein osteoglycin, referred to as osteoinductive factor (OIF), was first found as a stimulator of ectopic bone development. OIF has important functions in both lipid and glucose metabolism and is integrated into the typical vascular matrix. OIF has therefore been recently hypothesised to play an essential part in the glomerular disease related to nephropathy caused by diabetes, and it has also been investigated as an indicator for earlier DN [5].

The work's aim was to clarify the significance of OIF as a sensitive indicator for the detection of early diabetic nephropathy.

Patients and Methods

This observational prospective cohort work was done on 80 individuals 60 of them were diagnosed with T2DM according to ADA 2019 criteria.

The work was done following agreement from the Tanta University Hospitals' Ethical Committee, Egypt from May 2019 to March 2020. An informed authorization form was submitted by the participants.

Criteria for exclusion were participants with other diseases that may influence serum level of OIF as (osteoporosis, bone tumors, stress conditions including sepsis, hyperthyroidism, malignancy, pregnancy)

4 identical groups of patients formed: the first group (control group) consists of 20 people in good health. Participants with Type II diabetes with normo-albuminuria were in the second group. Participants with Type II diabetes with micro-albuminuria were in the third group. Participants with Type II diabetes with macro-albuminuria were in the fourth group All patients had accurate clinical and medical examinations, as well as laboratory tests.

Sampling

About 6.0 ml of venous blood was withdrawn (after about 10 to 12hr) fasting from each patient and control. 2.0 ml of blood was put into ethylene diaminetera acetic acid (EDTA) vacutainer tube for estimation of glycated Hb. The rest of blood was put into plain vacutainer tube, blood was allowed to coagulate then centrifuged it for 20 minutes (at 2000 - 3000 RPM), sera were used for estimation of fasting blood glucose level, renal function tests and lipid profile. The remaining sera were stored at -20℃ till the time of OIF assay, 2hs postprandial blood sample was withdrawn for estimation of post prandial blood glucose, early morning midstream urine was collected for urinary albumin and creatinine estimation.

Specific investigations

Serum level of OIF: by Human Osteoglycin (OGN/OIF) ELISA kit (SunRed, shanghai). Catalogue NO. 201-12-4462.

Test principle

The kit utilises a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) for assessing the concentration of Human Osteoglycin (OGN/OIF) in specimens subsequently it was incorporated into a monoclonal antibody enzyme thoroughly which is coated prior to use with Human Osteoglycin (OGN/OIF) monoclonal antibody, incubation; subsequently its antibodies labelled with biotin were included and mixed with Streptavidin-HRP to create an immune complex; afterwards incubating was performed and washing further to eliminate the separated enzyme. Following the addition of Chromogen Solutions A and B, the liquid's colour changed to blue before eventually becoming yellow as a result of the acid's effects. The amount of the human substance osteoglycin (OGN/OIF) in the specimen and the colour chroma are positively associated.

The distilled water was used to dilute the washing solution 30 times. Just chromogen solutions A and B, together with stop solution, were put to the blank well. For standard wells, 50 µL of streptavidin-HRP and 50 µL of standard were administered. Test wells: 40 µL of specimen was included, followed by 50 µL of streptavidin-HRP and 10 µL of OGN/OIF antibody. After being gently shacked and maintained for 60 minutes at 37 °C, the sealing membrane was formed. Each well received 50 µL of chromogen solution and 50 µL of chromogen solution B, which were then gently combined and maintained for 10 min at 37°C in the absence of light. The reaction was stopped by adding 50 µL of the stop solution into each well (the blue quickly became yellow).

Final measurement: optical density (OD) was determined at 450 nm wavelength within 15 minutes following applying the stop solution, using blank wells as the baseline.

The equation of the standard curve linear regression was developed using the standards' concentration and the associated OD values, and the sample's OD values were then used to the regression equation to determine the corresponding sample's concentration.

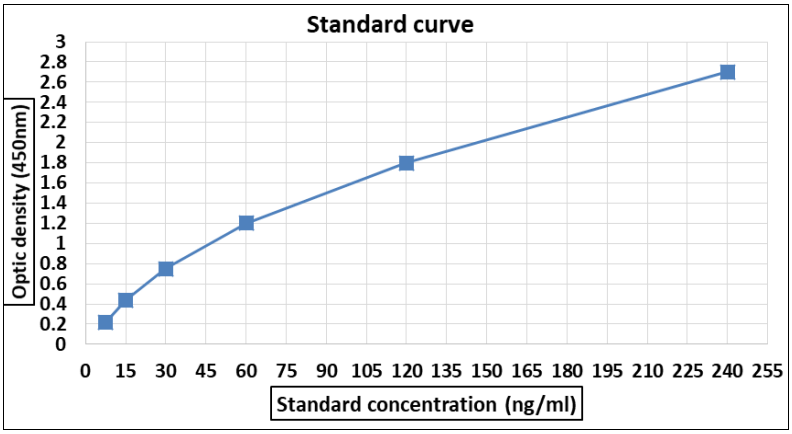


Fig 1: Optic density values

Statistical analysis

SPSS v26 (IBM Inc., Chicago, Illinois, USA) was used to conduct the statistical study. The ANOVA (F) test with the post hoc test (Tukey) was used to contrast quantitative parameters across each of the three groups. The quantitative variables were provided as the mean as well as the standard deviation (SD). The Chi-square test was used for analysing qualitative data, which were reported as both percentage (%) and frequency. To determine how closely two quantitative variables are correlated, Pearson correlation was used. Recover Operator Characteristics (ROC) curve analysis was utilized to evaluate the overall diagnostic performance of OIF. A two-tailed P value of >0.05 was considered statistically significant.

Results

There wasn't statistically significant variation among Four groups as regard age and gender with male predominance among all groups. While there was statistically significant variation between four groups as regard BMI as group II and III presented with larger BMI values in comparison to group IV and control group. Table 1

Table 1: Age, gender, BMI distribution among the studied groups.

		Group I (n =20)	Group II (n =20)	Group III (n =20)	Group IV (n =20)	Sig. test	P
Age (in years)		49.4 ± 3.7	49.4±3.9	50.7±3.08	49.9±2.6	F 0.596	0. 619
Sex	Male	15(75.0%)	16(80.0%)	18(90.0%)	15(75.0%)	F 1.988	0.617
	Female	5(25.0%)	4(20.0%)	2(10.0%)	5(25.0%)		
BMI (Kg/m^2)		22.7±1.6	24.0±2.3	24.5±2.4	22.8±1.7	F 3.889	0.012*

Data are presented as mean ±SD or frequency (%) BMI: body mass index *: statistically significant.

It was noticed greater mean values of fasting blood glucose, 2 hours post prandial blood glucose, and the haemoglobin A1c were statistically significantly correlated with greater levels of albuminuria across all variations, the higher the degree of albuminuria was associated with the higher mean values of serum total cholesterol, triglycerides and LDL with statistically significant difference for mentioned variants. HDL was higher in control group than all other groups. Table 2

Table 2: Blood glucose tests and lipid profile comparison among the groups under the study

		Group I	Group II	Group III	Group IV	Sig. test	P
Blood glucose tests	HbA1c%	5.0±0.5	7.8±0.5	8.2±0.6	8.5±0.6	164.069	0.001*
		P1	P2	P3	P4	P5	P6
		0.0001*	0.0001*	0.0001*	0.019*	0.0001*	0.082
	Fasting blood glucose (mg/dl)	89.9±1.5	173.0±8.0	180.2±16.0	202.9±23.5	223.429	0.001*
		P1	P2	P3	P4	P5	P6
		0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*
	2 hours post prandial blood glucose (mg/dl)	129.5±6.8	238.9±42.9	250.6±45.6	293.3±50.1	137.317	0.0001*
		P1	P2	P3	P4	P5	P6
		0.0001*	0.0001*	0.0001*	0.001*	0.0001*	0.0001*
Blood lipid profile	Total cholesterol (mg/dl)	186.0±7.2	195.7±3.7	201.3±4.0	204.2±6.5	41.638	0.0001*
		P1	P2	P3	P4	P5	P6
		0.0001*	0.0001*	0.0001*	0.002*	0.0001*	0.103
	Triglycerides (TG) (mg/dl)	126.6±13.6	177.6±12.0	181.6±11.1	265.8±40.7	126.290	0.0001*
		P1	P2	P3	P4	P5	P6
		0.0001*	0.0001*	0.0001*	0.583	0.0001*	0.0001*
	LDL (mg/dl)	115.3±8.9	122.7±9.9	126.5±7.5	144.1±13.9	28.83	<0.001*
		P1	P2	P3	P4	P5	P6
		0.027*	0.0001*	0.001*	0.249	0.001*	0.001*
	HDL (mg/dl)	45.1±6.0	37.6±8.6	38.4±6.7	39.9±7.1	4.455	0.006*
		P1	P2	P3	P4	P5	P6
		0.001*	0.004*	0.025*	0.709	0.303	0.510

Data are presented as mean ±SD or frequency (%) LDL: Low density lipoproteins HDL: high-density lipoprotein *: statistically significant.

Blood urea and serum creatinine significantly increased in group 4 in comparison to other groups also in eGFR in the 2 and 3 groups when contrasted to the 1 and 4 groups.

While a substantial reduction was existed in eGFR in group IV when contrasted to other groups for all mentioned variants. Regarding urinary albumin creatinine ratio There was substantial rise in the 4 and 3 groups when contrasted to both of the 1 and 2 groups, while no substantial difference was existed among group 1 and group 2. Table 3

Table 3: Serum creatinine, blood urea, e GFR and urine albumin creatinine ratio among the studied groups.

		Group I	Group II	Group III	Group IV	Sig. test	P
Renal functions	Serum creatinine (mg/dl)	1.0±0.1	1.1±0.1	1.2±0.2	2.1±0.7	42.324	0.001*
		P1	P2	P3	P4	P5	P6
		0.688	0.688	0.001*	0.422	0.001*	0.001*
	Blood urea (mg/dl)	23.5±4.4	32.9±6.8	37.2±8.1	82.9±32.2	47.897	0.001*
		P1	P2	P3	P4	P5	P6
		0.086	0.013*	0.001*	0.424	0.001*	0.001*
	eGFR %	112.4±3.7	137.8±4.2	134.6±5.3	73.7±8.1	560.262	0.001*
		P1	P2	P3	P4	P5	P6
		0.001*	0.001*	0.001*	0.074	0.001*	0.001*
Albumin creatinine ratio (mg albumin/gm creatinine)		19.3±4.5	24.4±4.4	116.5±59.8	429.1±60.0	530.0	0.001*
		P1	P2	P3	P4	P5	P6
		0.708	0.001*	0.001*	0.001*	0.001*	0.001*

Data are presented as mean ±SD or frequency (%) GFR: Glomerular filtration rate *: statistically significant.

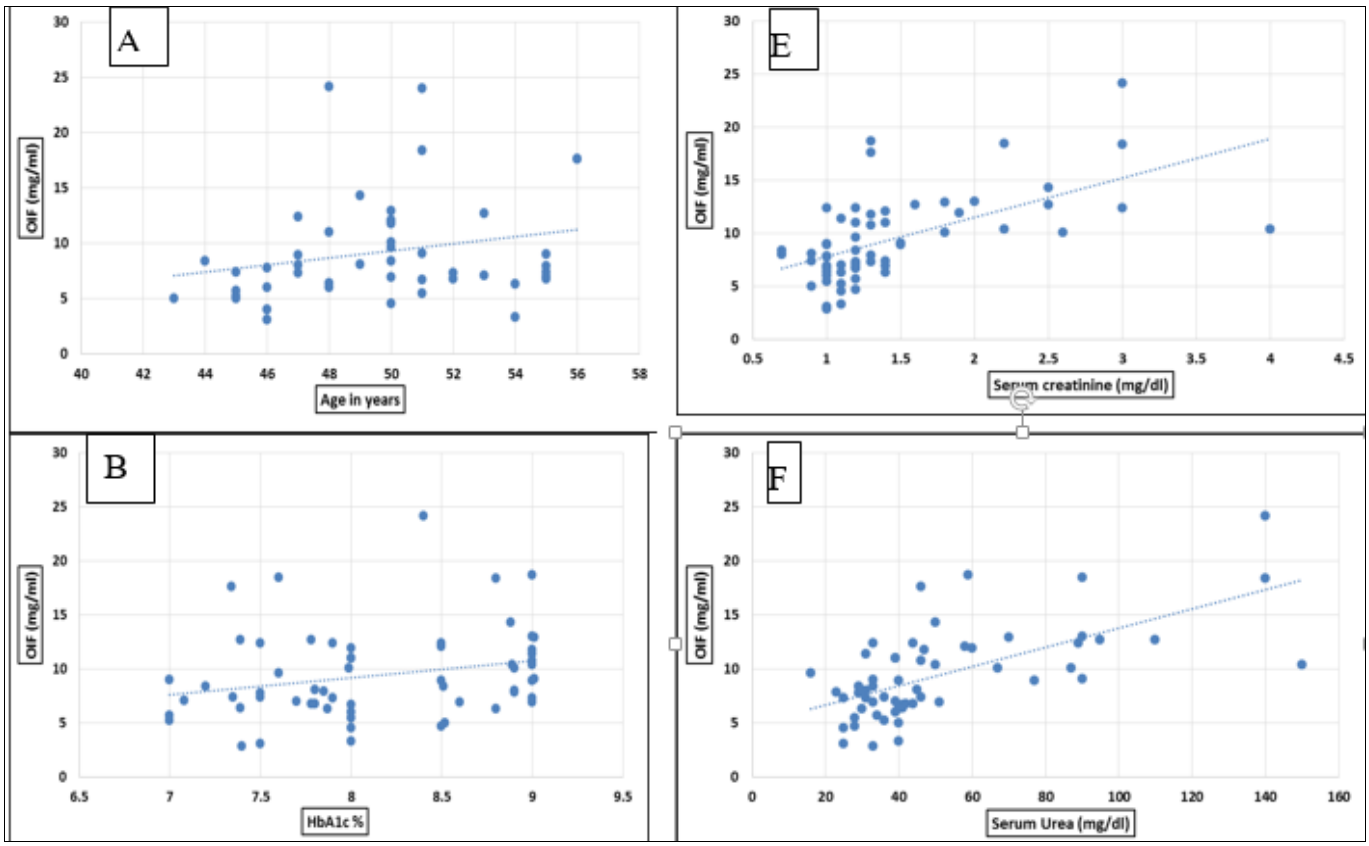
The degree of albuminuria was shown to be closely associated with OIF, with a statistically significant variance throughout the research groups showing an important increase in group 3 contrasted to 1 and 2 groups, but no substantial variation among 1 and 2 groups. Table 4

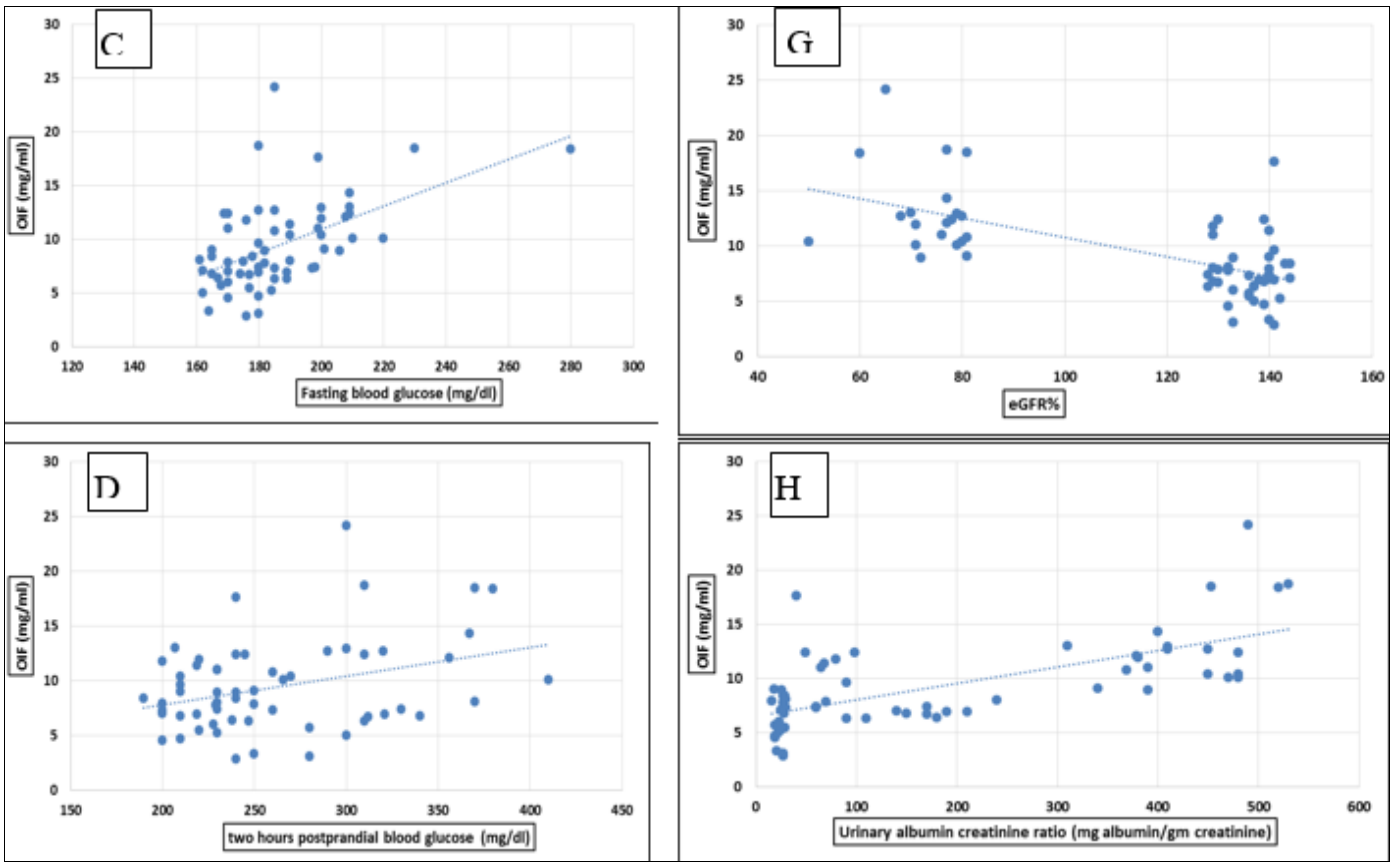
Table 4: OIF among the groups under the study:

	Group I	Group II	Group III	Group IV	Sig. test	P
OIF (mg/ml)	5.1±1.5	6.3±2.0	8.9±3.0	13.1±3.9	33.246	0.001*
	P1	P2	P3	P4	P5	P6
	0.193	0.0001*	0.0001*	0.004*	0.0001*	0.0001*

Data are presented as mean ±SD or frequency (%) OIF: Osteoinductive factor *: statistically significant.

There was significant positive association between OIF and HbA1c %, Fasting blood glucose level, 2 hours after meal blood Glucose, blood urea level, urinary albumin creatinine ratio, serum creatinine, Total cholesterol, TG and LDL, while there was negative correlation between OIF and both of eGFR and HDL (P.value < 0.05). Figure 2





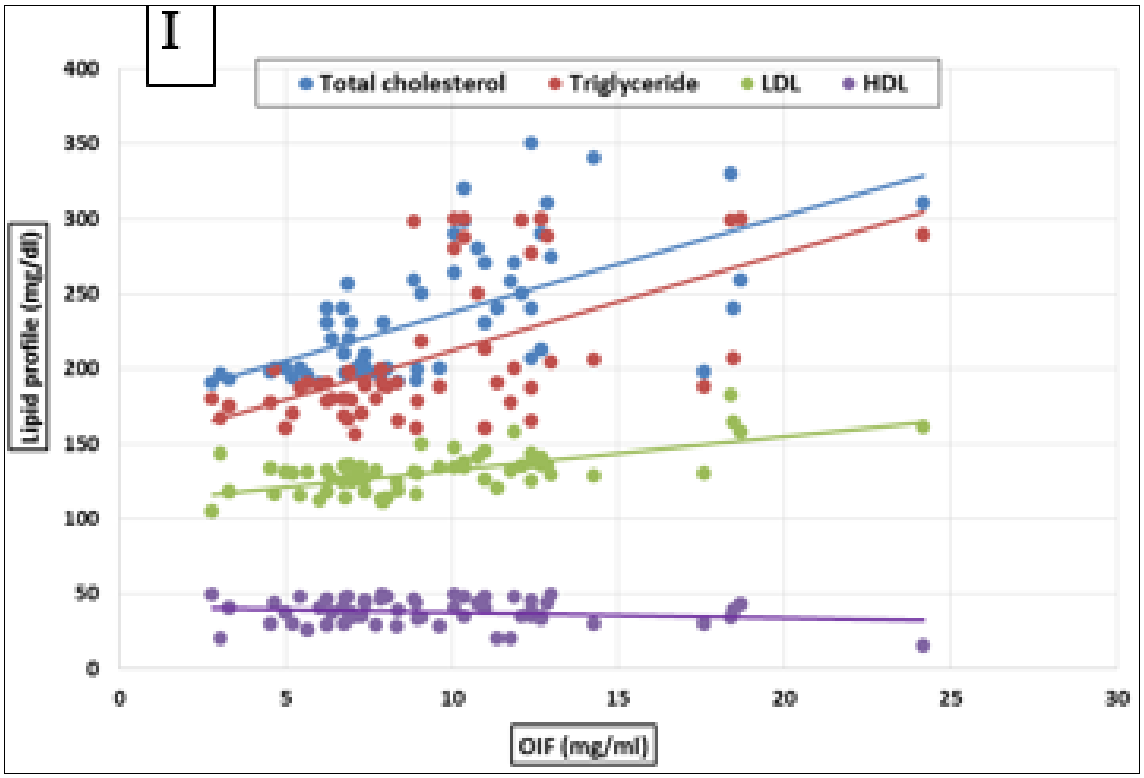


Fig 2: Correlation between(A) Age and OIF(B) HbA1c % and OIF (C) fasting blood glucose and OIF (D) hours postprandial blood glucose and OIF (E) serum creatinine and OIF (F) blood urea and OIF (G) eGFR % and OIF (H) urinary albumin creatinine ratio and OIF (I) lipid profile and OIF

Under the curve area (AUC) was 0.869 & p=0.014 indicating significant predictive ability of OIF for early detection of microalbuminuria. At cut-off 9.0, the sensitivity of OIF was 86.7% and the specificity was 95%. Figure 3

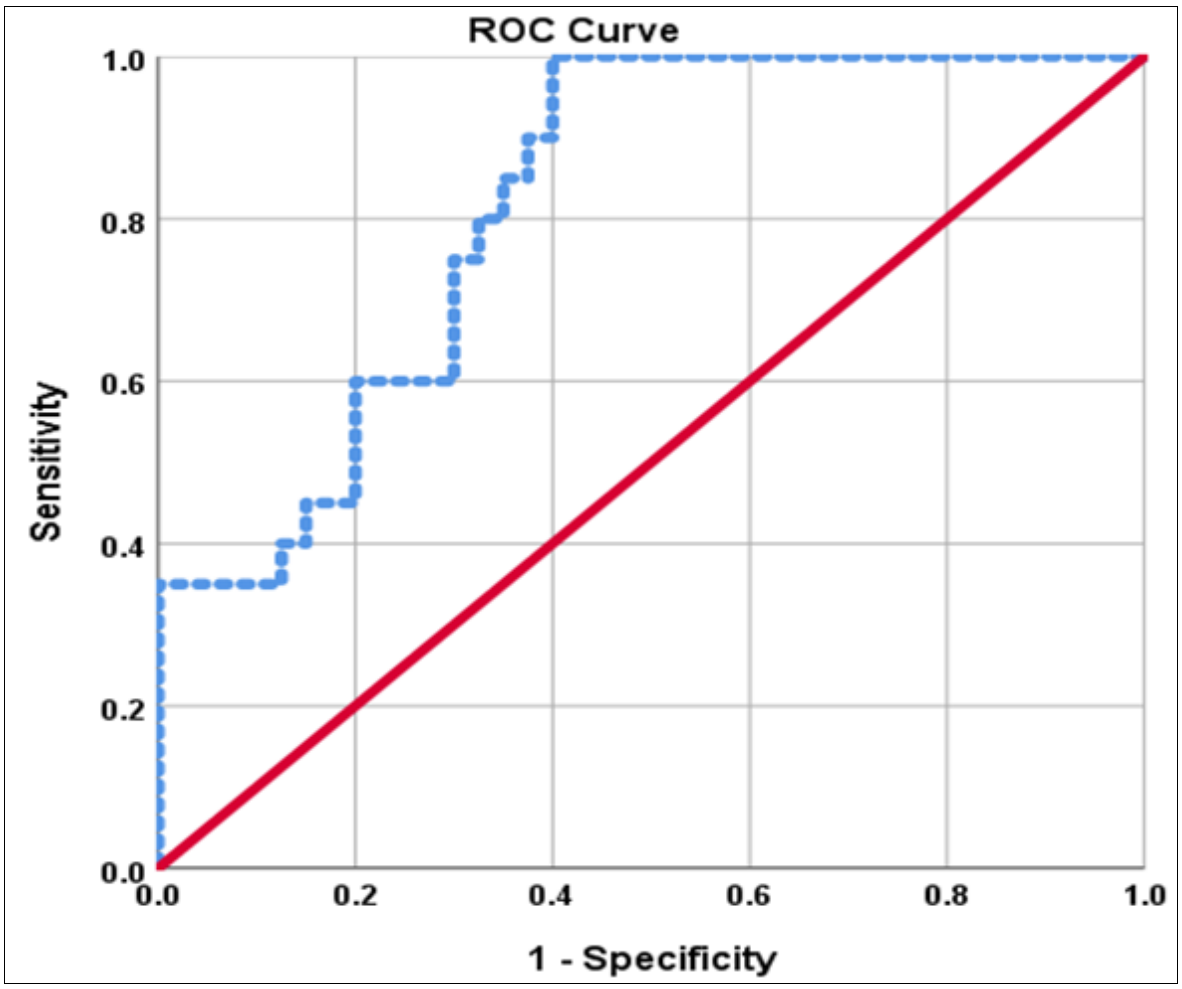


Fig 3: ROC curve analysis of OIF levels

Discussion

OIF contributes to the progression of atherosclerosis and angiogenesis. The pathophysiology and progression of DN include fundamental factors such as renal vessel atherosclerotic and vascular damage to endothelial tissue. According to reports, OIF does this function by interacting with bones morphogenetic proteins that resemble transforming growth factor (TGF)- [6]. Regarding OIF, this study showed that OIF directly proportionate to the degree of albuminuria with a statistically significant difference among the groups under the study. While no statistically substantial variance was existed among the control and normoalbumiuric groups, a major rise was existed in the micro-albuminuric group contrasted to both those groups. This was in approval with Wang, *et al*., [7] who found that OIF concentration was substantially greater in macro-albuminuric and micro-albuminuric individuals than in normo-albuminuric individuals and healthy people. Also Tamer A., *et al*., [8] suggested that, OIF was found in the early stages of DN, which is prior to microalbuminuria appeared, and it elevated as DN progressed.

In contrast, Wen W., *et al*.,[9], suggested that, serum OIF is inversely proportional to the degree of albuminuria, Additionally, they noted that the cause and decrease in serum osteoglycin (OGN) levels were significantly connected, indicating that Low concentrations of serum OGN could function as a standalone diagnostic indicator for DN linked to microalbuminuria in Type 2 diabetics.

In the present study, there was significant positive correlations among OIF and HbA1c %, fasting blood glucose, 2h p.p bl. glucose, serum creatinine, blood urea, urine albumin / creatinine ratio, total cholesterol, TG and LDL, while there was negative correlation between OIF and both of eGFR and HDL in T2DM patients (Normoal buminuric, microalbuminuric and macroalbuminuric groups).

These outcomes support the findings of Wang., *et al*., [7] who said that, there was an important positive relation between OIF and the same parameters as well, Tamer A., *et al*., [8], & González., *et al*., [10], showed that, there was a positive correlation between OIF and urinary albumin creatinine ratio, while there was a negative correlation between OIF and eGFR in diabetic patients. Tamer A., *et al*., [8], reported that OIF and creatinine exhibited a positive association whereas eGFR and OIF had a negative association. These results showed a strong correlation between OIF levels and deteriorating renal function in those with DN.

Oppositely, Wen W., *et al*., [9], they indicated that serum OIF is inversely proportionate to the degree of albuminuria and that OIF was positively connected with eGFR.

With regards to ROC curve analysis, our findings showed that serum OIF had a sensitivity of 86.7% and a specificity of 95% for identifying micro-albuminuria in Type 2 diabetes, with an AUC of 0.869 and a p-value of 0.014 at a cut-off of 9.0 ng/ml.

These findings confirmed those of Tamer A., *et al*. [8], who found that using ROC plots, serum OIF had a high specificity and sensitivity for the diagnosis of microalbuminuria. These findings suggested that serum OIF

levels may play a role in the progression of DN in people with diabetes.

It was recommended that serum OIF be used as an early indicator of nephropathy as it correlates with degree of albuminuria, further studies are recommended to assess serum OIF in DN understanding the complex connections between OIF, TGF-and bone morphogenetic proteins (BMPs) is also necessary.

Conclusions

Individuals with DN showed higher OIF serum concentrations. OIF is a sensitive indicator of early microalbuminuria and increases as diabetic nephropathy progresses. OIF is an important indicator of DN and is a reliable indicator of early nephropathy due to diabetes.

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Interest conflict: Nil

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