STUDY OF SERUM FERRITIN AND DIABETIC NEPHROPATHY IN TYPE 2 DIABETES IN EGYPTIANS

By

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ABSTRACT

Background: Diabetic nephropathy is a clinical syndrome characterized by persistent microalbuminuria in concomitance with diabetes mellitus. There is an urgent prerequisite for sensitive and/or more specific diagnostic and prognostic biomarkers for diabetic nephropathy serum ferritin levels reflecting the body iron stores, is known to be elevated in type 2 diabetes mellitus. However, its association with diabetic complications, including diabetic nephropathy (DN) and overall glycemic control, needs to be validated.

Objective: To identify and evaluate any association between elevated serum ferritin, glycemic control, microalbuminuria and other markers of diabetic nephropathy in patients with type 2 diabetes.

Patients and methods: The present study involved 80 patients who were receiving chronic care for type 2 diabetes at the outpatient clinics of Al-Obor Insurance Hospital during the period from January 2020 to March 2021. The selected subjects were divided into three groups: Group I included 20 healthy normal non-diabetic control subjects, age and sex matched. Group II included 40 patients who had type 2 diabetes mellitus without nephropathy, and Group III included 40 subjects who had diabetic nephropathy.

Results: The results of the present study revealed that both fasting and 2hPP blood glucose level was higher in diabetic patients with nephropathy compared to that detected in diabetic patients without nephropathy - and healthy controls and so for glycated hemoglobin (HbA1c) with mean value for diabetic patients with nephropathy, diabetic patients without nephropathy and healthy controls.

The results showed a progressively increase in the mean level of ferritin in diabetic groups. ANOVA test showed a statistically significant difference in the mean level of ferritin among different studied groups with F=24.971 and P<0.001. Post hoc test serum ferritin concentration increased significantly from control to diabetic without nephropathy groups, and from diabetic without nephropathy groups to diabetic nephropathy group (all P<0.05). There was a significant negative correlation between ferritin and eGFR. Pearson's correlation test revealed significant positive correlation between ferritin and each SGOT, SGPT, cholesterol, triglycerides, and LDL-c levels. However, there was no significant correlation between ferritin and HDL-c.

Conclusion: Patients with diabetes with higher serum ferritin were more likely to be associated with diabetic nephropathy. So, elevated serum ferritin level may be a useful marker for occurrence of nephropathy in diabetic patients.

Keywords: Serum ferritin, Diabetic Nephropathy, Type 2 diabetes.

INTRODUCTION

Chronic kidney disease (CKD) is one of the global public health problems. The heavy health and socioeconomic burden of end-stage renal disease (ESRD) underlines the importance of early screening for modifiable risk factors of CKD to prevent or delay the deterioration of renal function *(Wu et al., 2020)*. It has been estimated that more than 40% of people with diabetes will develop chronic kidney disease, including a significant number who will develop ESKD requiring renal replacement therapies (dialysis and or transplantation) *(Gheith et al., 2016)*. Early detection and better management of diabetic nephropathy (DN) patients with type 2 diabetes mellitus (T2DM) may delay DN progression to ESRD, lessen its complications, and improve outcome *(Zhao et al., 2020)*.

Ferritin is an index of body iron stores and is an inflammatory marker. Body iron stores are positively associated with the development of glucose intolerance, type 2 diabetes mellitus *(Renuka and Vasantha, 2016)*. Serum ferritin is an acute phase reactant, and is a marker of iron stores in the body. Iron is a transitional metal that can easily become oxidized and thus act as an oxidant. An important role of ferritin during the acute phase response is to restrict the availability of iron by sequestration into the cavity of the ferritin protein shell High body iron stores that is serum ferritin have been linked to insulin resistance, metabolic syndrome, and gestational diabetes *(Chiou and Connor, 2018)*.

Excess iron damages β-cells of pancreas due to oxidative stress which can contribute to pathogenesis of diabetes mellitus. In diabetic patient, the HbA1c may not be only correlated with blood sugar level, but also iron status if the patient happens to be suffering from iron deficiency anemia. Serum ferritin level had a relationship with hyperglycemia, and its level decreased with lowering of serum blood glucose *(Gandhi et al., 2018)*.

HbA1c or glycated hemoglobin, provides information about overall control of glucose in the previous 6-8 weeks, is considered the best available biochemical parameter to assess the long-term metabolic control in patients with DM. HbA1c levels are well associated with the response to treatment and hence act an important marker with which chances of developing complications in diabetics can be predicted. But, HbA1c may be affected by a variety of genetic, haematologic and illness-related factors *(Siddappa and Ramprasad, 2020)*.

Many factors lead to oxidative stress in CKD. Furthermore, ferritin also seems to be another factor contributing to oxidative stress in these patients. Studies about the possible link between oxidative stress and iron stores in patients on HD are important since high levels of iron can aggravate the oxidative stress already present in those patients *(Pedruzzi et al., 2015)*.

The aim of this study was to identify and evaluate any association between elevated serum ferritin, glycemic control, microalbuminuria and other markers of diabetic nephropathy in patients with type 2 diabetes.

PATIENTS AND METHODS

The study was designed as a case control study that was conducted during the period from January 2020 to March 2021 on 80 type 2 diabetes patients who were treated on an outpatient basis at Al-Obor Insurance Hospitable besides 20 age and sex matched normal apparent health were selected for the study.

The subjects were divided into 3 groups:

Group I: Twenty control subjects.

Group II: Forty type 2 diabetic patients without nephropathy.

Group III: Forty type 2 diabetic patients with nephropathy.

Inclusion criteria: Diagnosed type 2 diabetes mellitus patients on treatment, in the age group-45-65 years, and normal apparent health in the age group 45-65 years.

Exclusion criteria: Overt thyroid dysfunction, non-diabetic chronic kidney disease, chronic liver disease, on corticosteroid therapy, and hypertensive patients.

The following was done for patients and controls:

1. **Thorough history taking** including duration of DM.

2. **Clinical examination** for assessment of vital signs, chest examination, heart examination, abdominal examination, and neurological examination.

3. **Anthropometric measurements** for assessment of patients' weight, height then BMI was calculated using the formula: $BMI= weight (kg)/ [height (m)]^2$, and waist circumference (in cm).

4. **Routine investigations.**

5. **Laboratory measurements:** Biochemical tests for detection of blood glucose and kidney function tests were done on auto analyzer (AU680) from Beckman. The measurements included determination of glucose concentration, glycosylated hemoglobin A1c, serum cholesterol concentration, serum triglycerides concentration, serum HDL-cholesterol concentration, serum LDL-cholesterol concentration, urea, determination of serum and urine creatinine concentration, micro-albumin in urine, and calculation of estimated glomerular filtration rate (GFR).

Blood sampling and storage: Three millliters of venous blood sample were drawn from the antecubital vein using a disposable plastic syringe and left to clot for 30 minutes. The sample was then centrifuged, and serum was separated and kept at -20°C till assay time. Serum ferritin levels were measured by Enzyme Linked Fluorescent Assay (ELFA) technique.

Storage: Seven days at 2-8°C, or 2 weeks at -20°C.

Statistical Analysis:

In the present study, statistical analyses of data were carried out using SPSS version 23. Shapiro –Wilks test was used to test normal distribution of variables. Numerical data were expressed as mean ± standard deviation. Categorical data were summarized as percentages. The significance for the difference between groups was determined by using two-tailed Student's t test and Kruskal–Wallis H test & Post hoc test for quantitative data

as appropriate. Also Qualitative variables were assessed by Chi-squared (χ2) test.

The Receiver Operating Characteristic (ROC) was constructed to obtain the most sensitive and specific cutoff value for serum ferritin. Correlations between different parameters were done using Spearman's and Pearson's correlation write only the one you used coefficient and the area under the curve (AUC) greater than 0.5 was considered to be statistically significant. The probability (P) values of ≤0.05 were considered statistically significant.

RESULTS

The majority of cases in this study (n= 39, 39%) were in the sixth decade of life. There was only 1 patient had an age below 45 years and no patients had an age above 70 years of age. The mean age of controls was 55.9±6.97 years. Males represented 55% (n=11) of the controls whereas females represented 45% (n=9) of them. The mean age of diabetic patients with and without nephropathy was 56.2±6.48, and 56.28 ±7.12 years respectively. The percentages of males in the previously mentioned two groups were 55%, and 57.5% respectively. There was no statistical significant difference of age among all the studied groups. Also, a high percentage of males was shown in all studied groups but without statistically significant different between different groups (P>0.05) **(Table 1)**.

Table (1): **Mean Age and gender for different studied groups (control subjects (group I) and diabetic patients (groups II, III)**

Groups Parameters		GI (No.=20)	GII (No.=40)	GIII (No.=40)	Test of significance
Age (Yrs.)	Mean ± SD	55.9±6.96	56.28±7.12	56.2±6.48	Kruskal Wallis test Chi-Square = 0.028 P=0.986
	Range	46-64	42-65	45-65	
Gender Male Female		No. (%) 11 (55%) 9 (45%)	No. (%) 23 (57.5%) 17(42.5%)	No. (%) 22 (55%) 18(45%)	χ^2=0.061 P=0.970

- No.: Number of cases; Yrs: Years; SD: Standard deviation

Fasting and 2hPP blood glucose level was higher in diabetic patients with nephropathy (288.43 ± 99.05 & 338.43 ± 102.56 mg/dl) compared to that detected in diabetic patients without nephropathy – (216.43 ± 96.61 & 281.03 ± 136.04) and healthy controls 113.15±7.64, 151.55±16.58 (mg/dl) (P<0.001) and so for glycated hemoglobin (HbA1c) with mean value (8.59 ± 1.33 vs. 7.7 ± 1.068 vs. 5.74 ± 0.447) for diabetic patients with nephropathy, diabetic patients without nephropathy and healthy controls (P<0.001) **(Table 2)**.

Table (2): Comparison between the different studied groups as regards glucose and glycated hemoglobin (HbA1c)

Groups / Variables	(GI) (N=20)	GII (N=40)	GIII (N=40)	Kruskal Wallis test
Fasting blood glucose (mg/dl)				Kruskal Wallis test Chi-Square = 54.879 P<0.001
Mean ± SD	113.15 ± 7.64	216.43 ± 96.6[a]	288.43 ± 99.05	
2hPP blood glucose(mg/dl)				Kruskal Wallis test Chi-Square = 47.620 P<0.001
Mean ± SD	151.55 ± 16.58	281.025 ± 136.04 [a]	338.43 ± 102.56 [b]	
HbA1c (per gm %)				Kruskal Wallis test Chi-Square = 47.689 P<0.001
Mean ± SD	5.74 ± 0.45	7.7 ± 1.07 [a]	8.59 ± 1.33 [b]	

- a: significant difference from Healthy controls, b: significant difference from diabetic patients without nephropathy.

The mean value ± SD for the hemoglobin was (12.37±0.9, 11.38 ± 1.06, and 10.63 ± 1.02) (g/dL) in healthy controls, diabetic patients without nephropathy, and diabetic patients with nephropathy respectively. These results revealed that there was a significant decrease in the mean value of hemoglobin in diabetic patients with nephropathy when compared to those without nephropathy and healthy controls (P<0.001). Also hemoglobin level decreased in patients suffering from diabetic patients without nephropathy compared to its levels in healthy controls, but without significant difference (P<0.001).

The occurrence of diabetes rose in accompany with ESR elevation. ESR values after one hour were significantly higher in patients with diabetes either with or without nephropathy compared to healthy controls (56.13 ± 11.98 mm/h and 49.53± 12.76 versus 26.55 ± 3.87 mm/h, P< 0.001) after one hour and (68.78 ± 13.93 mm/h and 63.58 ± 14.78 versus 45.35 ± 4.2mm/h, P < 0.01) after 2 hours **(Table 3)**.

The mean levels of GOT in controls, diabetic patients without nephropathy and diabetic patients with nephropathy were 50.9±9.23, 58.33±8.13 and 63.95±14.82 (U/L), respectively and that of GPT were 40.3±5.37, 45.98±8.09 and 53.3±15.2 (U/L), respectively. The ANOVA test showed statistical significant difference in the mean level of both sGOT and sGPT among different studied groups with F=8.777, P<0.001 and F=9.872, P<0.001, respectively.

The post-hoc test revealed that there was significantly increased in the mean level of serum GOT from control group to diabetic without nephropathy group and control group to diabetic with nephropathy group (all P<0.05), whereas, there were non-significance differences between the two groups who had diabetic disease (P>0.05). As for sGPT, the concentration increased significantly from control to diabetic nephropathy group (P<0.01) whereas, there were non-significance differences between controls and diabetic without nephropathy group (P=0.067) **(Table 3)**.

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Table (3): Comparison between hemoglobin and ESR after 1st, 2nd hours, the serum GOT and GPT among control diabetic groups.

Groups / Variables	(GI) (N=20)	GII (N=40)	GIII (N=40)	Kruskal Wallis test
Hb (g/dl)				Kruskal Wallis test Chi-Square = 30.093 P<0.001
Mean ± SD	12.37±0.9	11.38±1.06 [a]	10.63±1.02 [ab]	
ESR 1st hour (mm/h)				Kruskal Wallis test Chi-Square = 51.694 P<0.001
Mean ± SD	26.55 ± 3.87	49.53± 12.76 [a]	56.13 ± 11.98 [ab]	
ESR 2nd hour (mm/h)				Kruskal Wallis test Chi-Square = 44.895 P<0.001
Mean ± SD	45.35 ± 4.2	63.58 ± 14.78 [a]	68.78 ± 13.93 [a]	
sGOT (U/L)				Kruskal Wallis test Chi-Square = 15.288 P<0.001
Mean ± SD	50.9±9.23	58.33±8.13 [a]	63.95±14.82 [ab]	
sGPT (U/L)				Kruskal Wallis test Chi-Square = 20.923 P<0.001
Mean ± SD	40.3±5.37	45.98±8.09	53.3±15.2 [ab]	

- a: significant difference from Healthy controls, b: significant difference from diabetic patients without nephropathy.

The mean urea, creatinine and Alb/Cr ratio were 33.4 ± 5.46, 0.89 ± 0.22 (mg/dl), 23.45 ± 5.26 (mg/g); 34.15 ± 6.21, 1.03 ± 0.26 (mg/dl), 97.47 ± 83 (mg/g); and 55.3 ± 18.78, 1.63 ± 0.67 (mg/dl), 931.95 ± 248.28 (mg/g); in group I, group II, and group III respectively. Individuals with diabetic nephropathy in comparison to diabetic patients without nephropathy and healthy controls showed a greater means of urea, creatinine, and Alb/Cr ratio (p<0.001). However, no statistically significant change was found in the mean of the previously mentioned markers when diabetic patients without nephropathy compared to healthy controls (p>0.05).

As regards eGFR, results revealed that patients with diabetic nephropathy had statistically significant lower mean GFR (50.53 ± 26.77) (mL/min) compared to both control subjects and diabetic patients without nephropathy (107.3 ± 10.39 & 98.2 ± 15.36) (mL/min) respectively, P≤0.001) **(Table 4)**.

The results showed that the mean levels of cholesterol, triglycerides and LDL-c were higher in diabetic patients with nephropathy (250.8 ± 81.55, 231.9 ±62.85, and 221.35 ±59.28 mg/dL respectively) and in diabetic patients without nephropathy (220.83 ±63.19, 225.05±64.68 and 225.5 ±62.78 mg/dL) than in healthy controls (172.3 ±37.43, 192.65±35.17 and 168.55 ±48.31 mg/dL with (P<0.05). Moreover, cholesterol level was the higher in diabetic patients with nephropathy compared to those without nephropathy (250.8 ± 81.55 vs. 220.83 ±63.19, p =0.05). However, there was no significant difference between all studied groups as regard to HDL levels (P=0.402) **(Table 4)**.

Table (4): Comparison between the different studied groups as regards the urinary markers, Triglycerides, Cholesterol, HDL-c and LDL-c.

Groups / Variables	(GI) (N=20)	GII (N=40)	GIII (N=40)	Statistical test
Urea (mg/dl)				Kruskal Wallis test Chi-Square = 38.325 P<0.001
Mean ± SD	33.4 ± 5.46	34.15 ± 6.21	55.3 ± 18.78 [ab]	
Creatinine (mg/dl)				Kruskal Wallis test Chi-Square = 31.154 P<0.001
Mean ± SD	0.89 ± 0.22	1.03 ± 0.26	1.63 ± 0.67 [ab]	
Alb/Cr ratio (mg/g)				Kruskal Wallis test Chi-Square = 83.913 P<0.001
Mean ± SD	23.45 ± 5.26	97.47 ± 83	931.95 ± 248.28 [ab]	
eGFR (mL/min/1.73 m^2)				Kruskal Wallis test Chi-Square = 57.693 P<0.001
Mean ± SD	107.3 ± 10.39	98.2 ± 15.36	50.53 ± 26.77 [ab]	
Cholesterol (mg/dl)				Kruskal Wallis test Chi-Square = 23.903 P<0.001
Mean ± SD	172.3 ± 37.43	220.83 ± 63.19 [a]	250.8 ± 81.55 [a,b]	
Triglycerides (mg/dl)				Kruskal Wallis test Chi-Square = 9.941 P=0.007
Mean ± SD	192.65 ± 35.17	225.05 ± 64.68 [a]	231.9 ± 62.85 [a]	
LDL-c (mg/dl)				Kruskal Wallis test Chi-Square = 18.799 P<0.001
Mean ± SD	168.6 ± 48.3	225.5 ± 62.78 [a]	221.35 ± 59.29 [a,]	
HDL-c (mg/dl)				Kruskal Wallis test Chi-Square = 2.733 P=0.255
Mean ± SD	40.25 ± 8.47	39.4 ± 8.11	41.93 ± 8.68	

- a: significant difference from Healthy controls, b: significant difference from diabetic patients without nephropathy.

The results showed that there was a statistically significant increase in the mean level of serum ferritin of diabetic patients.

The results showed progressively increase in the mean level of ferritin in diabetic groups, where the mean levels of ferritin in diabetic patients with nephropathy and those without nephropathy were 590.25±279.58 and 481.3±259.77 481.3±259.77 (ng/mL) respectively compared to controls of 123.95±52.07 (ng/mL). The ANOVA test showed statistically significant difference in the mean level of ferritin among different studied groups with F=24.971 and P<0.001.

Post hoc test for serum ferritin concentration increased significantly from control to diabetic without nephropathy groups and from diabetic without nephropathy groups to diabetic nephropathy group (all P<0.05) **(Table 5)**.

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Table (5): Mean of ferritin among control and different groups of diabetic patients

Groups / Variables	(GI) (N=20)	GII (N=40)	GIII (N=40)	Statistical test
Ferritin (ng/mL)				Kruskal Wallis test Chi-Square = 44.135 P<0.001
Mean ± SD	123.95±52.07	481.3±259.77 [a]	590.25±279.58[ab]	

a: significant difference from Healthy controls, b: significant difference from diabetic patients without nephropathy.

Pearson's correlation test showed positive significant correlation between ferritin and ESR after 1st and 2nd hours (r=0.561, P<0.001 and r=0.548, P<0.001, respectively). In contrast, negative correlation but not statically significant was observed between ferritin and age (r=-0.025, P=0.802 and Hb levels (r=-0.167, P=0.097).

Pearson's correlation test showed moderate significant positive correlation between ferritin and both fasting and 2hPP glucose levels as well as HbA1C (r=0.607, P<0.001, r=0.608, P<0.001, and r=0.604, P<0.001 respectively). Also, there was a week significant positive correlation between ferritin and urea, creatinine, and Alb/creatinine ratio (r=0.281, P=0.005, r=0.255, P=0.01, and r=0.386, P<0.001 respectively). On the other hand, significant negative correlation was found between ferritin and eGFR (r=-0.271, P=0.006).

Pearson's correlation test revealed significant positive correlation between ferritin and each sGOT, sGPT, cholesterol, triglyceride, and LDL-c levels (r=0.246, P=0.014, r=0.224, P=0.025, r=0.480, P<0.001, r=0.327, P=0.001, and r=0.393, P<0.001 r, respectively). However, there was no significant correlation between ferritin and HDL-c (r=-0.077, P=0.447) **(Table 6)**.

Table (6): Correlation of serum ferritin with age, Hb level, ESR, serum glucose, HbA1C, urea, creatinine, Alb/Cr, eGFR, sGOT, sGPT, and lipid profile

Ferritin (ng/mL) / Parameters	Pearson's correlation (r)	P-value
Age (Years)	-0.025	0.802
Hb(g/dL)	-0.167	0.097
ESR 1st hour	0.561	<0.001
ESR 2nd hour	0.548	<0.001
Fasting blood glucose (mg/dl)	0.607	<0.001
2hPP blood glucose(mg/dl)	0.608	<0.001
HbA1c (per gm %)	0.604	<0.001
Urea (mg/dl)	0.281	0.005
Creatinine (mg/dl)	0.255	0.01
Alb/Cr ratio (mg/g)	0.386	<0.001
eGFR	-0.271	0.006
sGOT	0.246	0.014
sGPT	0.224	0.025
Cholesterol (mg/dl)	0.480	<0.001
Triglycerides (mg/dl)	0.327	0.001
LDL-c (mg/dl)	0.393	<0.001
HDL-c (mg/dl)	-0.077	0.447

The ROC plots to assess the diagnostic efficiency of serum ferritin for detection of patients with diabetes from healthy controls. ROC curve analysis showed that serum ferritin had significantly higher diagnostic accuracy in diagnosis of diabetes disease (P<0.001). ROC curve showed the optimum cutoff for ferritin was 163 (ng /ml) for distinguishing patients with diabetes from healthy controls with sensitivity 95% and specificity 90%; an area under the ROC curve (AUROC) 0.968(95% CI: 0.936 - 1.000) **(Figure 1)**.
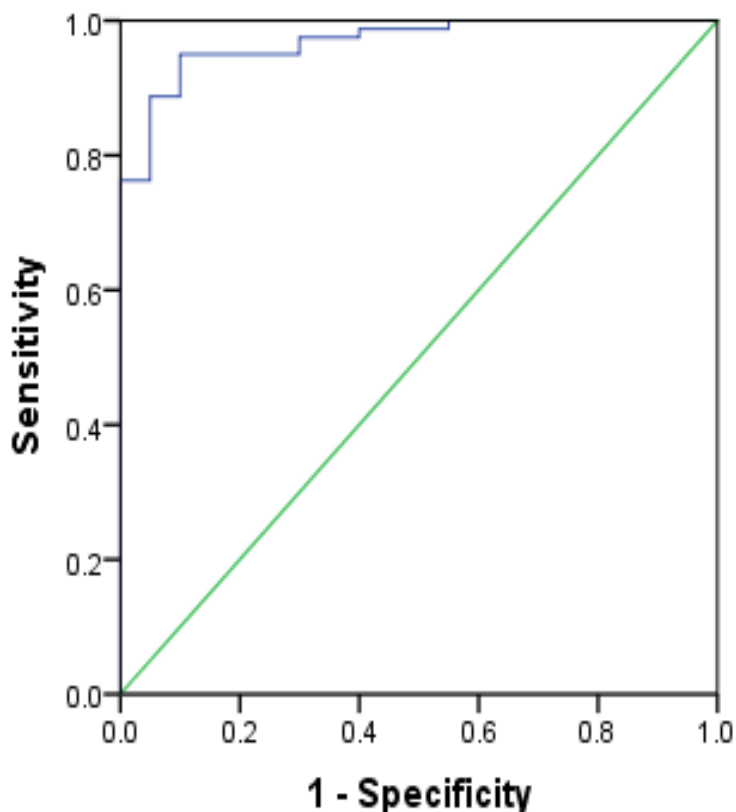

Figure (1): Nonparametric receiver operating characteristic (ROC) curves of ferritin for distinguishing patients with diabetes from healthy controls

The current study shows hyperferritinemia may be an independent risk factor of nephropathy in patients with Type 2 diabetes. ROC curve analysis showed that serum ferritin had significant diagnostic accuracy in diagnosis of diabetic patients with nephropathy from those without nephropathy (P=0.045). The best ferritin cutoff for differentiating diabetes patients with nephropathy from those without was 524 (ng /ml) with sensitivity 60% and specificity 72.5%; an area under the ROC curve (AUROC) 0.630(95% CI: 0.506 -0.755) **(Figure 2)**.
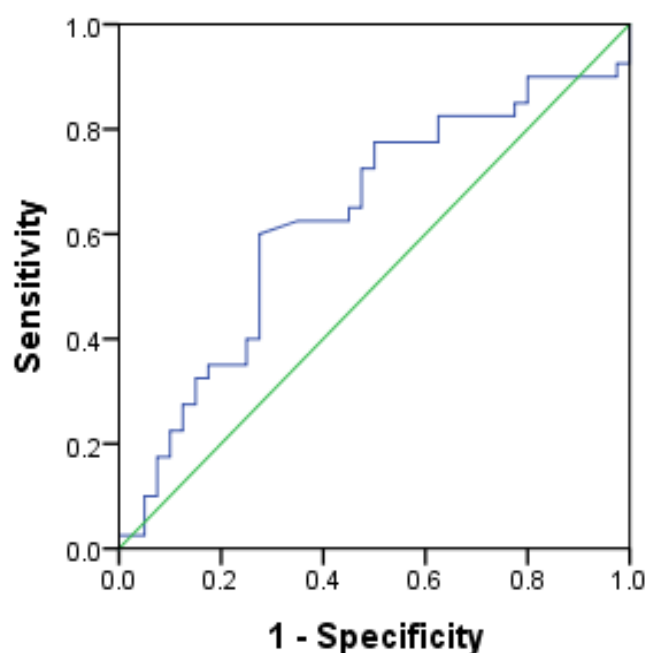
Figure (2): Nonparametric receiver operating characteristic (ROC) curves of ferritin for distinguishing patients with diabetic nephropathy from diabetic patients without nephropathy

DISCUSSION

Results of the current study revealed that the mean age of controls was 55.9±6.97 years. The males represented 55% of the controls whereas females represented 45% of them. The mean age of diabetic patients with and without nephropathy was 56.2±6.48, and 56.28 ±7.12 years respectively. The percentages of males in the previously mentioned two groups were 55%, and 57.5% respectively. There was no statistical significant difference of age among all the studied groups. Also, a high percentage of males was shown in all studied groups but without statistically significant different between different groups.

Older adults are at high risk for both diabetes and prediabetes, with surveillance data suggesting that half of older adults have are prediabetic *(Kirkman et al., 2012).*

The prevalence of diabetes is high in older subjects, affecting more than 20% of subjects > 65 years. Russo et al. (2018) on a large sample of outpatients with T2DM with a wide age-range showed that renal complications affect 41.3% of this population, and more than 60% of those aged > 75 years.

Neugarten and Golestaneh (2013) mentioned that male gender is a risk factor for progression of diabetic nephropathy. They supposed that sex hormones may affect numerous cellular processes directly or indirectly by modulating various cytokines synthesis or instance; growth factors, and vasoactive agent.

The effect of gender on diabetic renal disease is much more debatable. Even as a number of studies show that male gender is a risk factor for diabetic kidney disease (DKD) *(Goldberg and Krause, 2016).* Other studies demonstrated the reverse

that women are at higher risk of developing the ailment *(Zoppini et al., 2012)* and others found no significant discrepancy between men and women in terms of risk *(Monti et al., 2010)*.

Alrawahi et al. (2012) stated that a meaningful association is present between diabetic nephropathy and the following factors; poor glycemic control (high HbA1c), male gender, prolonged diabetes duration, hypertension, neuropathy, retinopathy, family history of diabetic nephropathy, and hypertriglyceridemia.

The results of the present study revealed that both fasting and 2hPP blood glucose level was higher in diabetic patients with nephropathy compared to that detected in diabetic patients without nephropathy, and healthy controls, and so for glycated hemoglobin (HbA1c).

These findings were in agreement with the previous studies that proved that bad glycemic control is a well-known risk factor of DN and a strong association is present between an increased levels of glycosylated hemoglobin (HbA1c) and increased mortality in patients with both diabetes and CKD *(Alrawahi et al., 2012, Yadav, 2012* and *Satirapoj & Adler, 2015). Siddappa and Ramprasad (2020)* reported that type 2 DM patients with nephropathy have poor glycemic control compared to them without nephropathy as evidenced by higher levels of HbA1c in DN group.

These results revealed that there was a significant decrease in the mean value of hemoglobin in diabetic patients with nephropathy when compared to those without nephropathy and healthy controls. Also hemoglobin level was decreased in patients suffering from diabetic patients

without nephropathy compared to its levels in healthy controls, but without significant difference.

It is reasonable to experience renal anemia in DKD. However, prior to its progression to DKD, patients with DM may experience hematologic disorder (increased erythropoiesis) *(Tsai and Tarng, 2019)*.

Hasslacher (2011) mentioned that in both sexes, the average haemoglobin levels decreased with decreasing kidney function (coefficient of correlation according to Pearson 0.305). According to WHO criteria, 44% of the women and 40% of the men with renal insufficiency revealed anaemia. A severe anaemia, i.e. haemoglobin values <11g/dl, was determined in 26% (female) and 17% (male) of the patients.

In addition, as expected individuals with diabetic nephropathy in comparison to diabetic patients without nephropathy and healthy controls showed a greater means of urea, creatinine, and Alb/Cr ratio. However, no statistically significant change was found in the mean of the previously mentioned markers when diabetic patients without nephropathy compared to healthy controls. As regards eGFR, results revealed that patients with diabetic nephropathy had statistically significant lower mean GFR compared to both control subjects and diabetic patients without nephropathy.

The post-hoc test revealed that there was a significantly increased in the mean level of serum sGOT from control group to diabetic without nephropathy group and control group to diabetic with nephropathy group, whereas, there were non-significance differences between the

two groups who had diabetic disease. As for sGPT the concentration increased significantly from control to diabetic nephropathy group whereas, there were non-significance differences between controls and diabetic without nephropathy group.

Elevated activities of serum aminotransferases are a common sign of liver disease and are observed more frequently among people with diabetes than in the general population. Many studies have shown an association between specific diabetic complications and disturbances in various tissues, such as diabetic nephropathy and cardiovascular diseases, but only limited data are available on the possible association between diabetic complications and liver function *(Arkkila et al., 2010)*.

In agreement to our results, *Adiga and Malawadiin (2016)* found a significant elevation in Total Bilirubin (TB), direct bilirubin (DB), aspartate amino transferase (AST) and alanine amino transferase (ALT) levels in diabetics was noted. An elevated eGFR and a significant correlation between eGFR and liver enzymes were observed. A significant association between liver and renal disease has been obtained in diabetics.

The results showed that the mean levels of cholesterol, triglycerides and LDL-c were higher in diabetic patients with nephropathy and in diabetic patients without nephropathy than in healthy controls. Moreover, cholesterol level was the higher in diabetic patients with nephropathy compared to those without nephropathy. However, there was no

significant difference between all studied groups as regard to HDL levels.

These results were keeping on with *Ali et al. (2017)* who found that there was a reduction in GFR levels in the macroalbuminuric and microalbuminuric diabetic groups compared to the control and normoalbuminuric diabetic groups. Likewise, *Kolhar and Priyanka (2017)* found significant association of DN with high TC, high LDL-C and high triglyceride.

This was in line with *Ejuoghanran et al. (2011)* who stated that altered lipid profile characterized by elevated triglyceride rich lipoproteins is present even in the early stages of the DN.

Moreover, the results showed progressively increase in the mean level of ferritin in diabetic groups, was significant difference in the mean level of ferritin among different studied groups with F=24.971. In Post hoc test serum ferritin concentration increased significantly from control to diabetic without nephropathy groups and from diabetic without nephropathy groups to diabetic nephropathy group.

In agreement to our findings, *Momeni et al. (2015)* suggesting that increased ferritin is an important finding in diabetes mellitus irrespective of the disease type.

Borah and Goswami (2016) have reported that type 2 diabetes mellitus is associated with increased serum ferritin, and that this finding may have a major role in the development of diabetes and its complications.

Similar results have also been reported by *Siddappa and Ramprasad (2020)* found that serum ferritin (SF) levels were

found to be increased in both type 2DM and DN compared to control group.

In line to our results, *Metwalley et al. (2020)* reported that serum ferritin levels were significantly higher in patients with T1DM compared with healthy individuals. The increase in serum ferritin concentrations was more evident in patients with microvascular complications than in those without these complications or in healthy controls. Also, the ferritin levels were found to influence microvascular complications independently of other risk factors.

Moreover, our results showed positive significant correlation between ferritin, ESR after 1st and 2nd hours, sGOT, sGPT, cholesterol, triglyceride, and LDL-c levels. Moderate significant positive correlation between ferritin and both fasting and 2hPP glucose levels as well as HbA1C. Also, there was a week significant positive correlation between ferritin and urea, creatinine, and Alb/creatinine ratio. In contrast, negative correlation but not statically significant was observed between ferritin and age and Hb levels.

On the other hand, significant negative correlation was found between ferritin and eGFR Pearson's correlation test revealed significant positive correlation between ferritin and each sGOT, sGPT, cholesterol, triglyceride, and LDL-c levels. However, there was no significant correlation between ferritin and HDL-c.

Association between the iron overload and glycemic control in diabetic patients has also been demonstrated in several studies like *Raj and Rajan (2013)* who reported a strong positive correlation between SF and HbA1 levels is found in these studies. Similar finding is observed in *Siddappa and Ramprasad (2020)* study where positive correlation between SF and HbA1c in diabetic nephropathy patients and a low positive correlation between the same parameters in diabetic patients without nephropathy signify the prognostic role of serum ferritin levels in diabetic patients.

Our findings reported that ROC curve showed the optimum cutoff for ferritin was 163 (ng /ml) for distinguishing patients with diabetes from healthy controls with sensitivity 95% and specificity 90%; an area under the ROC curve (AUROC) 0.968 (95% CI: 0.936 - 1.000). Moreover, serum ferritin had significant diagnostic accuracy in diagnosis of diabetic patients with nephropathy from those without nephropathy (P=0.045). The best ferritin cutoff for differentiating diabetes patients with nephropathy from those without was 524 (ng /ml) with sensitivity 60% and specificity 72.5%; an area under the ROC curve (AUROC) 0.630(95% CI: 0.506 - 0.755).

This was nearly the same to that reported by *Metwalley et al. (2020)* who was the first to define a cutoff value of 163.6 ng/mL for ferritin level indicating the presence of microvascular complications, which can be anticipated at the time of diagnosis with a sensitivity of 92.1% and specificity of 93.4%. They suggested that ferritin has potential clinical usefulness in T1DM and may be considered an additional suitable, inexpensive, and adequate prognostic marker for reliably detecting microvascular complications in children and adolescents with T1DM.

CONCLUSION

Patients with diabetes with higher serum ferritin were more likely to be associated with diabetic nephropathy. So, elevated serum ferritin level may be a useful marker for occurrence of nephropathy in diabetic patients.

REFERENCES

1. **Adiga US, and Malawadi BN. (2016):** Association of diabetic nephropathy and liver disorders. Journal of Clinical and Diagnostic Research, 10(10): 5-9.

2. **Ali OM, Mahfouuz MH and Zidan RA. (2017):** Assessment of circulating fibroblast growth factor 23 in type 2 diabetic patients with nephropathy. International Journal of Pharma and Bio Sciences, 8(3):1-12.

3. **Alrawahi AH, Rizvi SGA, Al-Riyami D and Al-Anqoodi Z. (2012):** Prevalence and risk factors of diabetic nephropathy in omani type 2 diabetics in Al-dakhiliyah region. Oman Medical Journal, 27(3): 212-218.

4. **Arkkila PE, Koskinen PJ, Kantola IM, Rönnemaa T, Seppänen E and Viikari JS. (2010):** Diabetic complications are associated with liver enzyme activities in people with type 1 diabetes. Diabetes Research and Clinical Practice, 52(2): 113-118.

5. **Borah M and Goswami RK. (2016):** Evaluation of serum ferritin in in type II diabetes mellitus: a hospital based observational study from Dibrugarh, Assam, India. International Journal of Research in Medical Sciences, 4(11): 4916-4921.

6. **Chiou B and Connor JR. (2018):** Emerging and dynamic biomedical uses of ferritin. Pharmaceuticals, 11(4): 124-130.

7. **Ejuoghanran OSO, Chukwu OE and Christopher SL. (2011):** The effect of diabetic nephropathy on the lipid profile of diabetics in Southern Nigeria. Journal of Medical Sciences, 11: 198-202.

8. **Gandhi SJ, Chaudhari AS, Pratinidhi SA and Sontakke AN. (2018):** Study of serum ferritin and HbA1c in type 2 diabetes mellitus. International Journal of Clinical Biochemistry and Research, 5(4): 594-598.

9. **Gheith O, Farouk N, Nampoory N, Halim MA and Al-Otaibi T. (2016):** Diabetic kidney disease: worldwide difference of prevalence and risk factors. Journal of Nephropharmacology, 5(1): 49-55.

10. **Goldberg I and Krause I. (2016):** The role of gender in chronic kidney disease. European Medical Journal, 1(2): 58-64.

11. **Hasslacher C. (2011):** Anaemia in patients with diabetic nephropathy—prevalence, causes and clinical consequences. European Cardiology Review, 3(1):80–82.

12. **Kirkman MS, Briscoe VJ, Clark N, Florez H, Haas LB, Halter JB and Swift CS. (2012):** Diabetes in older adults. Diabetes Care, 35(12): 2650-2664.

13. **Kolhar U and Priyanka P. (2017):** Study of serum lipid profile in type 2 diabetes mellitus patients and its association with diabetic nephropathy. International Journal of Advanced Medicine, 4(6): 1513-1516.

14. **Metwalley KA, Raafat DM, Tamer DM, Farghaly HS and Said GM. (2020):** Ferritin levels in children and adolescents with type 1 diabetes mellitus: relationship with microvascular complications and glycemic control. Archives of Endocrinology and Metabolism, 64(6): 720-725.

15. **Momeni A, Behradmanesh MS, Kheiri S and Abasi F. (2015):** Serum ferritin has correlation with HbA1c in type 2 diabetic patients. Advanced Biomedical Research, 4:74-78.

16. **Monti MC, Lonsdale JT, Montomoli C, Montross R, Schlag E and Greenberg DA. (2010):** Familial risk factors for microvascular complications and differential male-female risk in a large cohort of American families with type 1 diabetes. The Journal of Clinical Endocrinology and Metabolism, 92(12): 4650-4655.

17. **Neugarten J and Golestaneh L. (2013):** Gender and the prevalence and progression of renal disease. Advances in Chronic Kidney Disease, 20(5): 390-395.

18. **Pedruzzi LM, Cardozo LFF, Medeiros RF, Stockler-Pinto MB and Mafra D. (2015):** Association between serum ferritin and lipid peroxidation in hemodialysis patients. The Brazilian Journal of Nephrology, 37(2): 171-176.

19. **Raj S and Rajan GV. (2013):** Correlation between elevated serum ferritin and HbA1c in type 2 diabetes mellitus. Int J Res Med Sci., 1(1): 12-15.

20. **Renuka P and Vasantha M. (2016):** Study of the serum levels of iron, ferritin and magnesium in diabetic complications. International Journal of Pharmaceutical and Clinical Research, 8: 254-259.

21. **Russo GT, De Cosmo S, Viazzi F, Mirijello A, Ceriello A, Guida P and Fioretto P. (2018):** Diabetic kidney disease in the elderly: prevalence and clinical correlates. BMC Geriatrics, 18(1): 38-49.

22. **Satirapoj B and Adler SG. (2015):** Prevalence and management of diabetic nephropathy in western countries. Kidney Diseases, 1(1): 61-70.

23. **Siddappa MN and Ramprasad K. (2020):** Assessment of serum Ferritin level and its correlation with HbA1c in Diabetic Nephropathy. Asian Journal of Medical Sciences, 11(2): 46-51.

24. **Tsai SF and Tarng DC. (2019):** Anemia in patients of diabetic kidney disease. Journal of the Chinese Medical Association, 82(10): 752-755.

25. **Wu YH, Wang SY, Li MX, He H, Yin WJ, Guo YH and Zhang CH. (2020):** Serum Ferritin Independently Predicts the Incidence of Chronic Kidney Disease in Patients with Type 2 Diabetes Mellitus. Diabetes, Metabolic Syndrome and Obesity, 13: 99-105.

26. **Yadav SC. (2012):** Glycemic control in diabetic kidney disease patients. Clinical Queries: Nephrology, 1(2): 111-114.

27. **Zhao L, Zou Y, Zhang J, Zhang R, Ren H, Li L and Liu F. (2020):** Serum transferrin predicts end-stage Renal Disease in Type 2 Diabetes Mellitus patients. International Journal of Medical Sciences, 17(14): 2113-2124.

28. **Zoppini G, Targher G, Chonchol M, Perrone F, Lippi G and Muggeo M. (2012):** Higher HDL cholesterol levels are associated with a lower incidence of chronic kidney disease in patients with type 2 diabetes. Nutrition, Metabolism and Cardiovascular Diseases, 19(8): 580-586.

دراسة مستوى الفريتين في الدم وإعتلال الكلى في مرضي السكري من النوع الثاني في المصريين

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خلفيـــة البحــث: داء الكلــي الســكري هــو متلازمــة مصــاحبة كمضــاعفات لمــرض السـكري. ومــن المعــروف أن مسـتويات الفيـريتين فــي الـدم التــي تعكـس مخـزون الحديـد فــي الجسـم، ترتفـع فـي مرضـي السـكري مـن النـوع الثـاني. ومـع ذلـك، يجـب التحقـق مـن صـحة إرتباطـه بمضـاعفات مـرض السـكري بمـا فـي ذلـك اعتلال الكلية السكري والتحكم العام في نسبة السكر في الدم.

الهـدف مـن البحـث: تحديـد وتقيـيم أي ارتبـاط بـين ارتفـاع الفيـريتين فـي الـدم، والـتحكم فـي نسـبة السـكر فـي الـدم، ووجـود الميكـرو البيـومين فـي البـول وغيرهـا من علامات اعتلال الكلية السكري في مرضى السكري من النوع الثاني.

المرضــي وطــرق البحــث: إشــتملت الدراســة الحاليــة علــى 80 مريضًــا كــانوا يتلقـون رعايـة مزمنـة لمـرض السـكري مـن النـوع الثـاني فـي العيـادات الخارجيـة بمستشـفى العبـور للتـأمين خـلال الفتـرة مـن ينـاير 2020 إلـى مـارس 2021 بالإضــافة إلــى 20 شخصــا كمجموعــة ضــابطة. وتــم تقسـيم الأشـخاص المختـارين إلــى ثـلاث مجموعــات: المجموعــة الأولــى ضـمت 20 شخصًــا يتمتعـون بصـحة جيـدة وغيـر مصـابين بمـرض السـكري، وضـمت المجموعـة الثانيـة 40 مريضًـا يعـانون مـن مـرض السـكري مـن النـوع الثـاني دون اعتـلال الكليـة، وضـمت المجموعـة الثالثـة 40 شخصًـا يعـانون مـن اعـتلال الكليـة السكري.

نتائج البحث: أظهرت نتائج الدراسة الحالية أن كلا من مستويات السكر الصائم ومستوى الجلوكوز في الدم بعد الإفطار بساعتين كان أعلى لدى مرضى السكري الذين يعانون من اعتلال الكلية مقارنة بالمرضى المصابين بمرض السكر بدون اعتلال الكلية. والمجموعة الضابطة, وكذلك للهيموجلوبين السكري في مرضى السكري الذين يعانون من إعتلال الكلية ومرضى السكري غير المصابين بإعتلال الكلية والمجموعة الضابطة. وقد أظهرت هذه النتائج وجود إنخفاضاً معنوياً في متوسط قيمة الهيموجلوبين لدى مرضى السكري المصابين باعتلال الكلية عند مقارنتهم بأولئك الذين لا يعانون من إعتلال الكلية والمجموعة الضابطة, كما إنخفض مستوى الهيموجلوبين عند مرضى السكري الذين لا يعانون من إعتلال الكلية مقارنة بمستوياته في المجموعة الضابطة، ولكن دون فرق معنوي. وأظهر الاختبار اللاحق أن هناك زيادة معنوية في متوسط مستوى انزيمات الكبد في الدم من المجموعة الضابطة إلى مرضى السكري بدون إعتلال الكلية والمجموعة الضابطة إلى مجموعة مرضى السكري مع إعتلال الكلية. كما أظهرت النتائج زيادة تدريجية في متوسط مستوى الفيريتين في مجموعات مرضى السكر حيث كان متوسط مستويات الفيريتين في مرضى السكري المصابين بإعتلال الكلية والذين لا يعانون من إعتلال الكلية على التوالي مقارنة مع الضوابط. وكانت هناك فروقاً ذات دلالة إحصائية في متوسط مستوى الفيريتين بين المجموعات المدروسة المختلفة. وفي الاختبار اللاحق زاد تركيز الفيريتين في الدم بشكل معنوي من المجموعة الضابطة إلى مرضى السكري غير المصابين بإعتلال الكلية ومن مرضى السكري غير المصابين باعتلال الكلية إلى مجموعة إعتلال الكلية السكري.

الاستنتاج: كان مرضى السكري الذين يعانون من إرتفاع نسبة الفيريتين في الدم أكثر عرضة للإصابة بإعتلال الكلية السكري. لذلك، قد يكون إرتفاع مستوى الفيريتين في الدم علامة ذات أهمية لحدوث إعتلال الكلية في مرضى السكري.

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