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General Medicine

# ASSOCIATION BETWEEN SERUM FERRITIN AND MICROALBUMINURIA IN TYPE 2 DIADETES MELLITUS

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**ABSTRACT** **Aims and Ojective**: To study the association between serum ferritin and microalbuminuria in Type 2 diabetes mellitus and their role as early markers of nephropathy.

**MATERIAL AND METHODS**: A parallel randomized group study was conducted over a period of one year. One hundred patients of Type 2 diabetes mellitus, on oral hypoglycemic agents or Insulin therapy or both without any previous history of cardiovascular/ renal/liver disease were enrolled to explore any association between serum ferritin and microalbuminuria.

All patients after setinclusion /exclusion criteriawere subjected to thorough history using standard questionnaire,anthropometric measurements and routine investigations. Morning Spot urine albumin creatinine ratio for microalbuminuriaand serum ferritin levels were assayed. All the data was analyzed with the help of computer software SPSS version 17.0 and Epi-info version 6.0 for windows. Logistic regression was used to identify association between serum ferritin and microalbuminuria in Type 2 diabetes.The patients were divided into 4 quartiles based on serum ferritin levels and we found there were statistically significant differences between the four ferritin quartiles of all the studied variables of the entire cohort except the gender which showed non-significant differences.

**Results**: In our study, 100 Type 2 diabetes mellitus patients (consisting 71 males and 29 females) with age group 45-65years (51.75±6.42) were evaluated Elevated serum ferritin was a strong and independent risk factor for microalbuminuria in patients with Type 2 diabetes. The microalbuminuria prevalence increases from (Q1) to (Q4). Compared with individuals in the lowest quartile, those in the fourth quartile were more likely to have microalbuminuria.Our study shows significant association with duration of diabetes mellitus, diastolic blood pressure and serum ferritin levels in patients with microalbuminuria. In the entire cohort, ferritin was significantly correlated with HbA1c ($r$ = 0.33, p = < 0.001), fasting blood sugar ($r$ = 0.29, p = < 0.001), random blood sugar ($r$ = 0.34, p = < 0.001), urine albumin creatinine ratio ($r$ = 0.50, p = <0.0001) and total leucocyte count ($r$ = 0.28, p = <0.001) and C-reactive protein($r$ = 0.25, p = 0.03). Microalbuminuria was also found to be significantly correlated with HbA1c ($r$ = 0.33, p = < 0.001), fasting blood sugar ($r$ = 0.29, p = < 0.001), random blood sugar ($r$ = 0.34, p = < 0.001), ferritin level ($r$ = 0.50, p = <0.0001), total leucocyte count ($r$ = 0.28, p = <0.001) and C-reactive protein($r$ = 0.25, p = 0.03).

**Conclusion**: High serum ferritin is associated with a cluster of cardiovascular risks such as the metabolic syndrome, insulin resistance, obesity and elevated inflammatory markers. Microalbuminuria is also a manifestation of subclinical systemic inflammation, so it is understandable that high serum ferritin is associated with microalbuminuria and can serve as marker of early nephropathy were microalbuminuria can't be estimated.

**KEYWORDS :** Microalbuminuria. Subclinical systemic inflammation. Ferritin and Nephropathy.

## INTRODUCTION

India leads the world with largest number of diabetic subjects earning the dubious distinction of being termed the "diabetes capital of the world". According to IDF Diabetes Atlas 2014, every 7 seconds 1 person dies from diabetes[1]. There were 4.9 million deaths in 2014. Prevelance of diabetes in the world 8.3% in 2014 i.e.382 million people and number of people with the disease is set to rise to beyond 592 million in less than 25 years, 46.3% remained undiagnosed. According to IDF 6th edition, prevelance of diabetes in South East Asia is close to one-fifth of all adults with diabetes in the world[2]. People with diabetes in developing countries face a greater threat from its complications than those in wealthier countries.Prevelance of nephropathy in Type 2 diabetes mellitus is highly variable, ranging from 5-20%(UKPDS 64)[3]. As a contributor to mortality, Diabetes mellitus is the 5th leading cause of death in the world and accounted for 4.8 million deaths worldwide in the year 2012. It is a major cause of illness and premature death (International Diabetes Federation, Nov 2012)[4].

### An Overview of Nephropathy in Type 2 Diabetes.

Keeping in view of the present scenario of diabetes and its complication, benefits of early screening and detection in DKD (Diabetic kidney disease) derive primarily from early intervention to retard of proteinuria and preserve GFR.

Prevalence of nephropathy in Type 2 diabetes mellitus varies in different population groups i.e. fairly low incidence in Caucasians and a very high incidence in Pima Indians (Cowie CC et al 1989)[5]. However, data suggests that the renal risk is currently equivalent in the both types of diabetes(Ritz E et al, 1999)[6]. Some of the most robust data relating to the development of diabetic nephropathy in a population of predominantly white patients with T2DM was reported from the United Kingdom Prospective Diabetes Study (UKPDS) (Adler AI et al 2003)[7]. At ten years following diagnosis, the prevalence of microalbuminuria, macroalbuminuria, and either an elevated plasma creatinine concentration (defined as 175 µmol/L [2.041mg/dL]) or requirement for renal replacement therapy was 25, 5,and 0.8 percent, respectively. The yearly rate of progression from diagnosis to microalbuminuria, from microalbuminuria to macroalbuminuria, and from macroalbuminuria to an elevated plasma creatinine concentration or renal replacement therapy was 2.0, 2.8, and 2.3 percent respectively.

Increasing evidence indicates that activated inate immunity and inflammation are major player in the pathogenesis of DKD .Several cytokines-chemokines are coming up as possible predictors of renal disease (CRP, TNF alpha, MCP 1 ,ICAM 1 IL 18 etc.)

Ferritin one of the key proteins that play an important role in regulating iron homeostasis ,however, growing evidence has shown that even moderately increased iron stores represented by high-normal ferritin concentrations are associated with diabetes. More recently, the results from prospective studies from Caucasian populations suggested that iron overload could predict abnormal glucose metabolism. The mechanism linking iron overload and diabetes is yet to be established. At least three possible explanations may account for elevated ferritin concentrations in patients with diabetes.

1) Elevated ferritin concentrations may represent elevated body iron stores.
2) Ferritin is also an acute-phase reactant and its elevation may reflect inflammation.

3) Delayed clearance of glycosylated ferritin in patients with diabetes may have led to the elevated ferritin concentrations (Ford ES et al, 1999)[8].Excess iron deposition in the liver may cause insulin resistance by interfering with the ability of insulin to suppress hepatic glucose production.

Serum Ferritin as a possible marker of Diabetic Nephropathy. Recently, a report showed a positive correlation between serum ferritin and iron deposition in tissues, which linearly increased with diabetes duration( Raj S et al, 2013)[9]. The serum ferritin elevation is regarded as an index of iron overload, which successively leads to a condition called hemochromatosis (Rajpathak S et al, 2006)[10]. Several studies showed association between hemochromatosis and T2DM (Adams PC et al, 2005)[11].

The elevated iron level oxidizes various bio- molecules such as nucleic acids, proteins and lipids, which may contribute to T2DM development by decreasing insulin secretion from pancreatic beta cells with concomitant increase of insulin resistance (Manson JE et al, 2004)[12].

Serum ferritin is also considered a marker of systemic inflammation (Gabay C et al, 1999)[13].Therefore, patients with diabetes with poor glycaemic control might be expected to have elevated levels of serum ferritin (Canturk Z et al, 2003)[14].

In diabetic microangiopathy, however, Canturk et al, 2003, reported a significant association between serum ferritin and diabetic retinopathy. iron accumulation in hepatocytes may interfere with the insulin extracting capacity of the liver (Niederau C et al, 1984)[15] and affect insulin synthesis and secretion in the pancreas. Iron excess probably contributes initially to insulin resistance and subsequently to decreased insulin secretion (Wilson JG et al, 2003)[16]. Iron has been implicated in the pathogenesis of diabetic nephropathy (Swaminathan S et al, 2007)[17].

Glycated proteins have a substantial affinity for the transition metals, and the bound metal retains redox activity and participates in catalytic oxidation (Qian M, 1998)[18], glycochelates form could be involved in the vascular complications of diabetes (Sullivan JL, 1981)[19]. Oxidative stress from factors such as hyperglycemia, advanced glycation end products, and hyperlipidemia further contribute to the availability of intracellular iron that can generate and viciously worsen oxidative stress and renal damage (Johnson WT et al, 1984)[20]. Urinary iron excretion is increased early in the course of diabetic renal disease (Nishiya K et al, 1996)[21].

The first clinical sign of renal dysfunction in patients with diabetes is generally microalbuminuria (a sign of endothelial dysfunction that is not necessarily confined to the kidney (Ritz E et al, 2003)[22].

Microalbuminuria develops in 2 to 5 percent of patients per year (Gall MA et al, 1997)[23]. Microalbuminuria is seldom reversible in Type 2 diabetes (Parving H et al, 2001)[24]. It progresses to overt proteinuria in 20 to 40 percent of patients (Nelson RG et al, 1991).[25]. In 10 to 50 percent of patients with proteinuria, chronic kidney disease develops that ultimately requires dialysis or transplantation (Brenner BM et al, 2001)[26] Although the mechanism linking iron concentrations and diabetes is yet to be established, it is known that iron is a catalyst in the formation of hydroxyl radicals, which may contribute initially to insulin resistance, subsequently to decreased insulin secretion, and ultimately to the development of Type 2 diabetes (Jiang R, Manson JE, Meigs JB et al 2004)[27].

Iron overload can cause cell damage through mechanisms involved in increase of oxidative stress, activation of inflammatory cytokines and macrophage infiltration (Wrede CE et al, 2006)[28]. Imanishi et al, 1999[29]demonstrated that glomerular hypertension is present in Type 2 diabetic patients with early nephropathy and closely correlated with increased urinary albumin excretion.

Renal endothelial function and related haemodynamics may be affected by a cascade of reactions caused by excess iron, of which high serum ferritin is indicative (Kim CH, Kim HK, Bae SJ et al, 2011)[30]

Furthermore, ferritin itself is often referred to as a marker of systemic inflammation which could be attributable to microalbuminuria. Microalbuminuria is also a manifestation of subclinical systemic inflammation (Barzilay JI, Peterson

D, Cushman M et al, 2004)[31], it is understandable that high serum ferritin was found to be associated with microalbuminuria. Patients with diabetes mellitus who exhibited higher serum ferritin were more likely to have microalbuminuria. Further investigation of the mechanisms by which ferritin may influence microalbuminuria development is needed (Y. H. Hsu, M. C. Huang, H. Y. Chang et al, 2013)[32].

**Aims and Ojective**

To study association between serum ferritin and microalbuminuria in Type 2 diabetes mellitus.To study serum ferritin and microalbuminuria as a markers of early nephropathy.

**Material and methods**

A parallel randomized group study was conducted in Post-Graduate Department of Medicine, Acharya Shri Chander College of Medical Sciences and Hospital, Sidhra, Jammu over a period of one year from 1st November 2013 to 31st October 2014. One hundred patients of Type 2 diabetes mellitus attending indoor or outdoor wings of Department of Medicine, on oral hypoglycemic agents or Insulin therapy or both without any previous history of cardiovascular/ renal/liver disease were studied to explore any association between serum ferritin and microalbuminuria in Type 2 diabetes mellitus. The study was approved by the Institutional Ethics and Board of Studies Committee of University of Jammu.

**INCLUSION CRITERIA:**

1. Patients fulfilling the criteria for the diagnosis of T2DM according to American Diabetes Association (ADA) guidelines or those on oral anti-diabetic medications/Insulin.
2. Age group 45-65 years.

**EXCLUSION CRITERIA:**

1. Overt thyroid dysfunction.
2. Chronic kidney disease.
3. Chronic liver disease.
4. Patients on chronic corticosteroid therapy.
5. Urinary tract infection.
6. Anaemia.

Evaluation of Patients: All subjects were explained the purpose of the study in the local language and a written informed consent was obtained as per rule.

All patients were subjected to thorough history especially regarding the following: diabetes, hypertension, smoking, heart disease, renal disease, liver disease using standard questionnaire and past drug history.

Anthropometric measurements: Height, Weight, BMI (Body Mass Index) according to the standard technique.

**Routine Investigations:**

- Fasting blood sugar
- Lipid profile- (S. cholesterol, S. triglycerides, HDL cholesterol, LDL cholesterol)
- Blood urea
- Serum. creatinine
- Total leucocyte count
- Serum ferritin : Chemiluminiscence
- C-reactive protein
- HbA1c levels
- Liver function tests
- Morning Spot urine albumin creatinine ratio for microalbuminuria

**Microalbuminuria :** Morning spot urine albumin creatinine ratio, quantitative measure of urine protein loss. Early morning first voided urine taken, we measured the albumin:creatinine ratio on morning spot urine, was found to be acceptable according to the Kidney Disease Outcomes Quality Initiative (KDOQI) Clinical Practice Guidelines. It may overestimate the prevalence of microalbuminuria.

**Method : Automatic**

All the data was analyzed with the help of computer software SPSS version 17.0 and Epi-info version 6.0 for windows. Logistic regression was used to identify association between serum ferritin and

microalbuminuria in Type 2 diabetes.

**Observations**

We divided the patients into 4 quartiles based on serum ferritin levels. Bivariate analysis was performed to study the correlation of levels with the studied variables, using Pearson Correlation coefficient.

**Table 1 Demographics and biochemical characteristics of study subjects by quartile of serum ferritin concentration**

| Variables | Ferritin quartile (Mean±SD) | | | | p-value |
|---|---|---|---|---|---|
| | <100 | 101-200 | 201-300 | >301 | |
| Age | 53.83±8.08 | 52.63±6.99 | 53.83±8.08 | 53.13±6.88 | <0.0001 |
| Male, n (%) | 8.0, (66.67) | 10.0, (62.5) | 28.0,(68.29) | 25.0,(80.65) | 0.2381 |
| Female, n (%) | 4.0, (33.33) | 6.0, (37.5) | 13.0,(31.71) | 6.0, (19.35) | |
| BMI | 26.76±2.66 | 25.00±2.99 | 26.76±2.66 | 26.23±3.73 | <0.0001 |
| SBP | 122.83±8.11 | 132.13±12.93 | 122.83±8.11 | 131.94±12.66 | <0.0001 |
| DBP | 78.67±8.71 | 79.88±9.11 | 78.67±8.71 | 81.68±10.09 | <0.0001 |
| DOD | 5.28±1.69 | 4.92±1.47 | 5.28±1.69 | 7.07±2.87 | 0.0014 |
| HbAIC | 7.27±1.16 | 7.56±1.68 | 7.27±1.16 | 8.84±1.34 | 0.0002 |
| BSF | 128.5±22.55 | 129.46±36.78 | 128.5±22.55 | 152.84±39.03 | 0.0002 |
| RBS | 176.17±65.18 | 210.56±48.72 | 176.17±65.18 | 250.81±72.59 | 0.0014 |
| UACR | 127.26±70.57 | 130.18±61.74 | 127.26±70.57 | 258.06±42.86 | 0.0158 |
| T.CHOL | 196.67±36.19 | 176.00±56.21 | 196.67±36.19 | 186.68±36.66 | <0.0001 |
| TG | 258.58±240.86 | 53.44±21.54 | 258.58±240.86 | 167.23±76.6 | 0.0323 |
| HDL | 48.33±30.12 | 81.38±45.06 | 48.33±30.12 | 43.83±26.3 | 0.0078 |
| LDL | 92.5±43.45 | 157.27±35.62 | 92.5±43.45 | 103.32±29.07 | 0.0056 |
| TLC | 6925±1454.85 | 6281.25±832.04 | 6925±1454.85 | 7919.35±1875.17 | 0.0002 |
| CRP | 4.55±5.4 | 3.08±2.91 | 4.55±5.4 | 8.25±13.44 | 0.0190 |
| Creatinine | 1.69±1.61 | 1.36±1.03 | 1.69±1.61 | 3.77±6.44 | 0.0310 |
| Rx, n (%) | | | | | |
| OHA | 9.0, (75.00) | 13.0, (81.25) | 38.0, (92.68) | 30.0, (96.77) | <0.0001 |
| Insulin | 1.0, (8.33) | 2.0, (12.50) | 3.0, (7.32) | 0.0, (0.00) | |
| OHA+Insulin | 2.0, (16.67) | 1.0, (6.25) | 0.0, (0.00) | 1.0, (3.33) | |

The data presented in Table 1 revealed that there were statistically significant differences between the 4 ferritin quartiles of all the studied variables of the entire cohort except the gender which showed non-significant differences. Highest significant mean age (53.83) was observed in the ferritin quartile of <100 and 201-300, while ferritin quartile 101-200 showed the lowest mean age 52.63±6.99.

**Table 2 Demographics of patients with Type 2 diabetes with and without progression to microalbuminuria**

| Variables | Without microalbuminuria | With microalbuminuria | p-value |
|---|---|---|---|
| Age | 52.2±6.8 | 48.38±7.27 | 0.13 |
| Male, n (%) | 6.0 (75) | 66.0 (71.74) | 0.83 |
| Female, n (%) | 2.0 (25) | 26.0 (28.26) | |
| BMI | 26.12±3.17 | 25.72±4.55 | 0.74 |
| SBP | 129.09±12.95 | 126.25±10.93 | 0.55 |
| DBP | 79.41±8.73 | 81.75±7.13 | 0.02 |
| DOD | 6.25±2.32 | 2.59±1.48 | <0.0001 |
| HbAIc | 8.3±1.68 | 7.4±0.67 | 0.14 |
| BSF | 147.87±39.92 | 131.71±23.03 | 0.26 |
| RBS | 228.38±66.96 | 197.38±94.94 | 0.23 |
| T.CHOL | 182.22±38.61 | 172.88±35.84 | 0.51 |
| TG | 167.98±99.85 | 200.75±166.89 | 0.40 |
| HDL | 45.52±22.13 | 41.5±17.3 | 0.62 |
| LDL | 99.92±34.1 | 91.38±40.6 | 0.50 |
| FERRITIN | 245.53±90.79 | 140.88±73.04 | 0.00 |
| TLC | 7194.57±1651.8 | 7587.5±1837.26 | 0.52 |
| CRP | 6.31±9.23 | 7.44±5.87 | 0.73 |
| Creatinine. | 2.18±4.03 | 1.46±1.57 | 0.62 |
| Rx, n (%) | | | |
| OHA | 7 (87.50) | 83 (89.12) | 0.19 |
| Insulin | 0 (0.00) | 6 (6.52) | |
| OHA+Insulin | 1 (12.50) | 3 (3.26) | |

Perusal of the Table 2 revealed that DBP, DOD and ferritin levels showed the significant differences with regard to the presence microabuminuria, while rest of the variables exhibited non-significant differences.

**Table 3: Comparison of Clinical features of type 2 diabetics with serum ferritin concentration**

| Variables | Female | Male | p-value |
|---|---|---|---|
| Gender | 29 | 71 | - |
| AGE | 52.24±7.99 | 51.75±6.42 | 0.75 |
| BMI | 25.74±3.61 | 26.23±3.14 | 0.49 |
| SBP | 129.79±14.28 | 128.48±12.2 | 0.64 |
| DBP | 79.79±6.58 | 79.52±9.35 | 0.88 |
| DOD | 6.11±2.69 | 5.9±2.39 | 0.70 |
| HbAIc | 8.07±1.6 | 8.3±1.66 | 0.53 |
| BSF | 144.28±41.1 | 147.52±38.38 | 0.71 |
| RBS | 217.62±67.86 | 229.28±70.35 | 0.45 |
| UACR | 186.71±85.42 | 179.4±90.77 | 0.71 |
| T.CHOL | 170.62±41.15 | 185.9±36.47 | 0.07 |
| TG | 149.97±48.41 | 179.04±121.14 | 0.21 |
| HDL | 51.28±22.34 | 42.72±21.14 | 0.07 |
| LDL | 93.24±41.95 | 101.69±30.97 | 0.27 |
| TLC | 6806.9±1513.26 | 7397.18±1697.73 | 0.11 |
| CRP | 4.84±5.84 | 7.04±9.96 | 0.27 |
| Creatinine | 2.31±3.79 | 2.05±3.95 | 0.76 |
| Rx, n (%) | | | |
| OHA | 24 (82.76) | 66 (92.96) | 0.20 |
| Insulin | 3 (10.34) | 3 (4.23) | |
| OHA+Insulin | 2 (6.90) | 2 (2.81) | |

Perusal of the Table 3 revealed that DBP, DOD and ferritin levels showed the significant differences with regard to the presence microabuminuria, while rest of the variables exhibited non-significant differences.

**Table 4: Correlation of variables with ferritin level**

| Variable | r-value | p-value |
|---|---|---|
| Age (years) | -0.02 | 0.82 |
| Body mass index (kg/m$^2$) | 0.06 | 0.52 |
| Systolic blood pressure (mm Hg) | 0.16 | 0.09 |
| Diastolic blood pressure (mm Hg) | 0.10 | 0.31 |
| Duration of diabetes (years) | 0.25 | 0.01 |
| Glycosylated hemoglobin (HbAIc) | 0.33 | 0.00 |
| Fasting blood sugar (mg/dl) | 0.29 | 0.00 |
| Random blood sugar (mg/dl) | 0.34 | 0.00 |
| Urine albumin creatinine ratio (mg/g) | 0.50 | <0.0001 |
| Total cholesterol (mg/dl) | 0.01 | 0.91 |
| Triglyceride (mg/dl) | -0.15 | 0.12 |
| High density lipoprotein | -0.16 | 0.11 |
| Low density lipoprotein | 0.11 | 0.26 |
| Total leucocyte count (mm$^3$) | 0.28 | 0.00 |
| C-reactive protein (mg/l) | 0.25 | 0.01 |
| Creatinine (mg/dl) | 0.21 | 0.03 |

Table-4:- Bivariate analysis was performed to study the correlation of levels with the studied variables, using Pearson Correlation coefficient. In the entire cohort, ferritin was significantly correlated with HbAIc ($r = 0.33$, $p = < 0.001$), fasting blood sugar ($r = 0.29$, $p = < 0.001$), random blood sugar ($r = 0.34$, $p = < 0.001$), urine albumin creatinine ratio ($r = 0.50$, $p = <0.0001$) and total leucocyte count ($r = 0.28$, $p = <0.001$). Correlation with C-reactive protein in the studied subjects was also statistically significant ($r = 0.25$, $p = 0.03$).

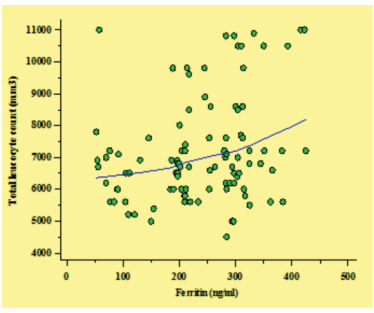



**Figure 1**

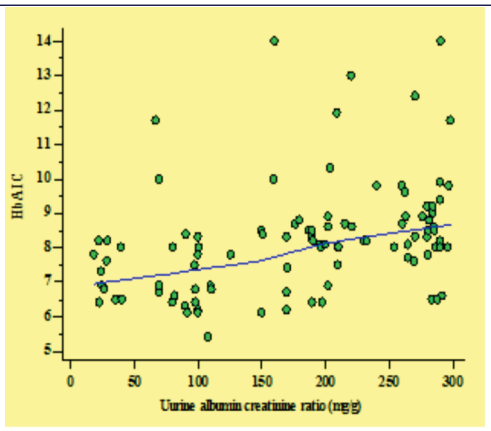



**Figure 2**

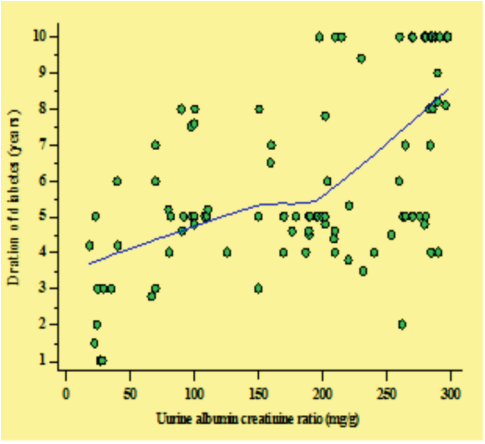



**Figure 3**

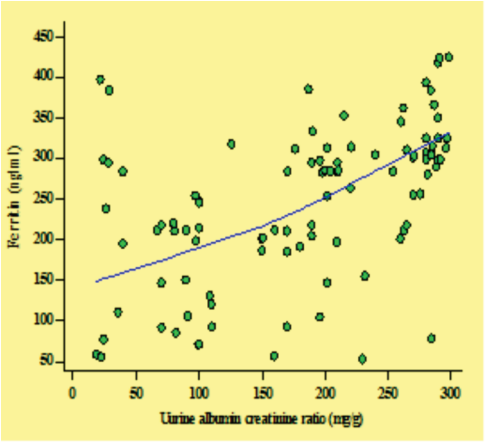



**Figure 4**

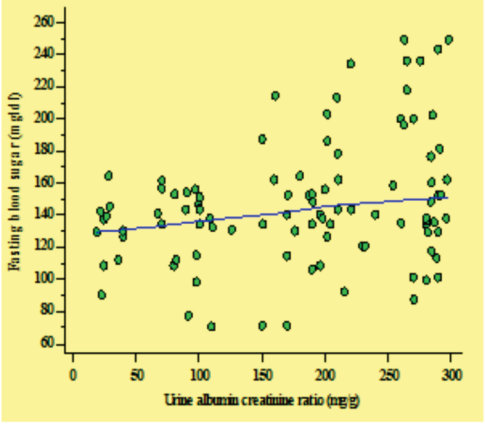



**Figure 5**

**Table 5. Correlation of variables with microalbuminuria**

| Variable | r-value | p-value |
|---|---|---|
| Age (years) | 0.26 | 0.00 |
| Body mass index (kg/m$^2$) | 0.10 | 0.31 |
| Systolic blood pressure (mm Hg) | 0.20 | 0.05 |
| Diastolic blood pressure (mm Hg) | 0.13 | 0.20 |
| Duration of diabetes (years) | 0.57 | <0.0001 |
| Glycosylated hemoglobin (HbAIc) | 0.38 | 0.00 |
| Fasting blood sugar (mg/dl) | 0.32 | 0.00 |
| Random blood sugar (mg/dl) | 0.41 | <0.0001 |
| Serum ferritin (ng/ml) | 0.50 | <0.0001 |
| Total cholesterol (mg/dl) | 0.11 | 0.28 |
| Triglyceride (mg/dl) | -0.09 | 0.34 |
| High density lipoprotein | -0.002 | 0.98 |
| Low density lipoprotein | 0.11 | 0.27 |
| Total leucocyte count (mm$^3$) | 0.14 | 0.15 |
| C-reactive protein (mg/l) | 0.13 | 0.19 |
| Creatinine (mg/dl) | 0.20 | 0.05 |

Table 5:-Bivariate analysis was performed to study the correlation of levels with the studied variables, using Pearson Correlation coefficient. In the entire cohort, serum ferritin was significantly correlated with HbAIc ($r = 0.33$, p = < 0.001), fasting blood sugar ($r = 0.29$, p = < 0.001), random blood sugar ($r = 0.34$, p = < 0.001), UACR ($r = 0.50$, p = <0.0001) and total leucocyte count ($r = 0.28$, p = <0.001). Correlation with C-reactive protein in the studied subjects was also statistically significant ($r = 0.25$, p = 0.03).

**Table 6: Overall Model Fit**

| | |
|---|---|
| Null model -2 Log Likelihood | 120.430 |
| Full model -2 Log Likelihood | 103.928 |
| Chi-squared | 16.503 |
| P-value | < 0.0001 |

**Table 7: Logistic Regression Predicting Decision from UACR**

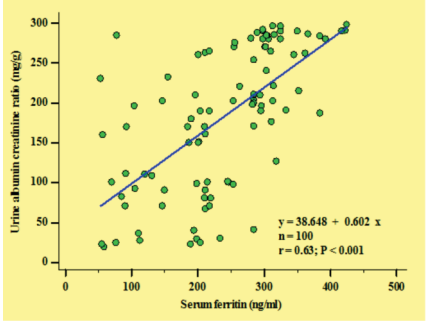
| Predictor | Coefficient | Std. Error | p-value | Odds ratio | 95% CI |
|---|---|---|---|---|---|
| UACR | 0.010742 | 0.0028625 | 0.0002 | 1.0108 | 1.0051 to 1.0165 |
| Constant | -0.8832 | | | | |

**Table 8: Contingency table for Hosmer & Lemeshow test**

| Group | Y=0 | | Y=1 | | Total |
|---|---|---|---|---|---|
| | Observed | Expected | Observed | Expected | |
| 1 | 5 | 6.428 | 5 | 3.572 | 10 |
| 2 | 5 | 5.213 | 5 | 4.787 | 10 |
| 3 | 5 | 4.533 | 5 | 5.467 | 10 |
| 4 | 6 | 3.653 | 5 | 7.347 | 11 |
| 5 | 3 | 2.459 | 7 | 7.541 | 10 |
| 6 | 2 | 2.114 | 8 | 7.886 | 10 |
| 7 | 1 | 1.573 | 9 | 8.427 | 10 |
| 8 | 1 | 1.260 | 10 | 9.740 | 11 |
| 9 | 1 | 1.208 | 11 | 10.792 | 12 |
| 10 | 0 | 0.560 | 6 | 5.440 | 6 |
| Chi-square | 4.3817 | | | | |
| p-value | 0.8211 | | | | |

**Table 9: Regression Equation**

| Parameter | Coefficient | Std. Error | 95% CI | T | p-value |
|---|---|---|---|---|---|
| Intercept | 30.2794 | 20.5308 | -8.4603 to 69.0190 | 1.4748 | 0.1435 |
| Slope | 0.6367 | 0.07163 | 0.4946 to 0.7789 | 8.8898 | <0.0001 |



A logistic regression analysis( Table7) was conducted to predict the association between serum ferritin and microalbuminuria in Type 2 diabetes A test of the null and full model( Table 6) were statistically significant, indicating that there was close association between serum ferritin and microalbuminuria in Type 2 diabetes (chi square = 16.503, p < 0.0001). Both ferritin and microalbuminuria levels were independent predictors of onset of diabetes (odds ratio [OR] 1.0108; 95% CI 1.0051 to 1.0165). ( Table7&8)

Regression equation indicated that if the ferritin level is increased by 1 (ng/ml) then UACR will increase approximately 0.602 (mg/g) and if the ferritin level is 0 (mg/g) then the model predicts that UACR will increase approximately 38.65 (mg/g). ( Table 9 Fig 6).

## Discussion

Diabetes mellitus is the commonest endocrine disorder. Systemic inflammatory activity plays a key role in the pathogenesis of diabetes related complications .Inflammatory biomarkers may be a valuable tool for risk evaluation. Among them best evidence to date supports the use of high-sensitivity C-reactive protein to monitor cardiovascular risk in diabetic and nondiabetic individuals (Pfutzner A, 2010 )[33]. High serum ferritin is associated with a cluster of cardiovascular risks such as the metabolic syndrome, insulin resistance, obesity and elevated inflammatory markers. Microalbuminuria is also a manifestation of subclinical systemic inflammation, so it is understandable that high serum ferritin was found to be associated with microalbuminuria. Main aim of our study to find any association between serum ferritin and microalbuminuria in Type 2 diabetes mellitus.

In our study, 100 Type 2 diabetes mellitus patients (consisting 71 males and 29 females) with age group 45-65years (51.75±6.42) were evaluated

We divided the patients into 4 quartiles based on serum ferritin levels and we found there were statistically significant differences between the 4 ferritin quartiles of all the studied variables of the entire cohort except the gender which showed non-significant differences. Elevated serum ferritin was a strong and independent risk factor for microalbuminuria in patients with Type 2 diabetes(Table 2). The microalbuminuria prevalence increases from (Q1) to (Q4). Compared with individuals in the lowest quartile, those in the fourth quartile were more likely to have microalbuminuria. (Table 2).

Our study shows significant association with duration of diabetes mellitus, diastolic blood pressure and serum ferritin levels in patients with microalbuminuria.

In the entire cohort, ferritin was significantly correlated with HbA1c ($r$ = 0.33, p = < 0.001), fasting blood sugar ($r$ = 0.29, p = < 0.001), random blood sugar ($r$ = 0.34, p = < 0.001), urine albumin creatinine ratio ($r$ = 0.50, p = <0.0001) and total leucocyte count ($r$ = 0.28, p = <0.001) and C- reactive protein($r$ = 0.25, p = 0.03). (Table 4). Microalbuminuria was also found to be significantly correlated with HbA1c ($r$ = 0.33, p = < 0.001), fasting blood sugar ($r$ = 0.29, p = < 0.001), random blood sugar ($r$ = 0.34, p = < 0.001), ferritin level ($r$ = 0.50, p = <0.0001), total leucocyte count ($r$ = 0.28, p = <0.001) and C-reactive protein($r$ = 0.25, p = 0.03). (Table 5).

Sushma B, Nagarajappa K, Mallkarjun CR (2013)[34] studied serum ferritin as a nobel risk factor for diabetes and mean serum ferritin was found to be higher in diabetes subjects compared to healthy and prediabetes subjects.

In our study, significant association was found between serum ferritin and diabetes mellitus.

Hsu Y H, Huang MC, Chang HY et al (2013)[35] found an association between serum ferritin and microalbuminuria in Type 2 diabetes in Taiwan. This study shows that hyperferritinemia may be an independent risk factor of nephropathy in patients with Type 2 diabetes. In this study, they found that those with higher ferritin concentrations were also more likely to have inferior metabolic profiles i.e. higher triglycerides (P < 0.001), lower HDL (P < 0.001), higher hsCRP (P < 0.05) and higher prevalence of microalbuminuria (P < 0.05). This was the first study to report an association between elevated serum ferritin and microalbuminuria in patients with Type 2 diabetes mellitus. In our study, ferritin was significantly correlated with HbA1c ($r$ = 0.33, p = < 0.001), fasting blood sugar ($r$ = 0.29, p = < 0.001), random blood sugar ($r$ = 0.34, p = < 0.001), urine albumin creatinine ratio ($r$ = 0.50, p = <0.0001) and total leucocyte count ($r$ = 0.28, p = <0.001) and C-reactive protein($r$ = 0.25, p = 0.03) (Table / Fig.1). Our study showed serum ferritin and microalbuminuria as independent markers of diabetic nephropathy. Our observations agree with the results reported by Hsu Y H et al, 2013[35].

Louise HD, Mary N et al (2013)[36]found in their study that the sex differences in the association between serum ferritin and fasting glucose in Type 2 diabetes mellitus among South Asian Surinamese, African Surinamese, and ethnic Dutch. In this population-based SUNSET study, they found a positive association between serum ferritin and Type 2 diabetes and fasting glucose in the multiethnic population, which appeared stronger among women than men. In our study, women were less in number and no significant difference was found from this study.

Sumesh R, G. V. Rajan (2013)[37] found correlation between elevated serum ferritin and HbA1c in Type 2 diabetes mellitus. Serum ferritin was significantly higher in diabetic patients when compared to controls and serum ferritin had a positive correlation with increasing duration of diabetes. This study showed positive correlation between serum ferritin and FBS, HbA1c and there was no correlation between serum ferritin and age, sex, metabolic syndrome, coexistent hypertension, total cholesterol, LDL and serum triglycerides. In our study, significant association was found between serum ferritin, FBS, RBS, HbA1c. Some of our results were concordant with this study.

Israa HI, Haider KZ, Qasim MA et al (2012)[38]studied the prevalence of microalbuminuria in Type 2 diabetes mellitus patients in Al-Husain Hospital in Karbala province of Iraq. In this study, the prevalence of microalbuminuria was high (59%). The risk factors that accompanied microalbuminuria were high blood pressure, elevated fasting blood glucose and poor glycemic control. Our results agree with the data reported by this study. (Fig 3&5).

Varghese A, Deepa R, Rema M, Mohan V (2001)[39] found the prevalence of microalbuminuria in Type 2 diabetes mellitus and found overall prevalence of microalbuminuria was 36.3% (95% confidence interval 33.8 to 38.9). The prevalence of microalbuminuria increased with the increase in duration of diabetes. In our study also microalbuminuria showed positive association with duration of diabetes (Fig 3).

Sumeet S & RP Kudyar (2008)[40]found a relationship between serum ferritin and Type-2 diabetes mellitus. In this observational study, a total of 50 obese Type-2 diabetic patients were selected randomly and were compared with an equal number of age, sex, BMI matched controls to find out correlation between serum ferritin and serum insulin levels. Influence of body iron stores on various biochemical parameters like HbA1c, total cholesterol(TC), triglyceride (TG), high density lipoprotein (HDL), uric acid (UA) and low density lipoprotein (LDL) as well as development on diabetic complications was also studied. Increased serum ferritin levels were found to be associated with increased serum insulin levels reflecting insulin resistance, poor glycemic control and complications of Type 2 diabetes mellitus. In our study, significant association was found between FBS, RBS, HbA1c levels and also as a marker of early nephropathy.

John et al (1991)[41] reported the prevalence of diabetic nephropathy 19.7% from a tertiary hospital in Vellore, south India and they found that the prevalence of diabetic nephropathy in non insulin dependent diabetes and reported male sex, older age, longer duration of diabetes, poor glycaemic control and raised blood pressure as risk factors of microalbuminuria. In our study significant association was found between microalbuminuria (marker of early nephropathy) with duration of diabetes and poor glycemic control.(Fig 3&5)

Gupta DK, Verma LK, Khosla PK et al (1991)[42] found the prevalence of microalbuminuria in diabetes and reported a prevalence of 26.6% microalbuminuria in 65 Type 2 north Indian non-proteinuric patients and also reported HbA1c to be associated with microalbuminuria. In our study significant association was found between microalbuminuria, HbA1c. (Fig 2 )

## Summary and conclusion

- In this study, significant association was found between duration of diabetes mellitus, diastolic blood pressure and serum ferritin

levels in patients with microalbuminuria.

- Serum ferritin was significantly correlated with HbA1c, fasting blood sugar, random blood sugar, urine albumin creatinine ratio, total leucocyte count and C-reactive protein.
- Microalbuminuria was significantly correlated with HbA1c, fasting blood sugar, random blood sugar, serum ferritin, total leucocyte count and C-reactive protein.

In this study, serum ferritin was found to be significantly associated with microalbuminuria in patients with Type 2 diabetes mellitus and as an independent marker of diabetic nephropathy. As many studies regarding microalbuminuria as a marker of early nephropathy are already done, serum ferritin as an independent marker of early nephropathy need further studies

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