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Serum Ferritin: Relationship with Indices of Obesity Among Patients with Type 2 Diabetes Mellitus in Kano, North-Western, Nigeria

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#### Abstract

Body iron stores have been shown to be significantly higher in persons with diabetes. We determined the relationship between serum ferritin and indices of obesity among type 2 diabetics attending the diabetes clinic of Aminu Kano Teaching Hospital (AKTH), Kano, North-Western Nigeria. We recruited 100 patients with type 2 diabetes attending the Diabetes clinic of AKTH. A pre-tested questionnaire was used to obtain data on socio-demography, duration of diabetes, treatment history and history of hypertension. Patients with liver disease, sickle cell anaemia or other haemoglobinopathies; any acute illness, malignancy and patients on iron or folate containing medications were excluded from the study. We measured anthropometric indices and blood pressure using standard methods. Laboratory investigations included serum ferritin, fasting plasma glucose and 2hour postprandial glucose. Data were analyzed using SPSS version 16 for windows and STATA version 11. A p value <0.05 was considered significant. Of the 100 patients, 40(40%) were males and 60(60%) were females. The mean (SD) values of BMI, WC, WHR and WhtR were  $26.3(5.9) \text{ kg/m}^2$ , 94.5(14.9) cm, 0.98(0.13) and0.6(0.1) respectively. The overall prevalence of obesity was 19.8%. The mean (SD) serum ferritin of the subjects was 79.8(88.1) ng/ml. There was negative correlation between BMI and log ferritin (r = -0.02, p = 0.01), while WHR correlated positively but not significantly with log ferritin (r=0.21, p=0.48). No correlation was found between log ferritin and duration of diabetes, gender, WC, WhtR, SBP, DBP, FBS and 2HPPG. Among the indices of obesity, only WHR

correlated positively with serum ferritin. There was no correlation between age, gender, duration of DM, SBP, DBP, FBS and 2HPPG and serum ferritin among our subjects. We recommend further studies in a larger sample on the subject to include variables such as insulin resistance and HbA1C.

**Keywords:** Indices of obesity, serum ferritin, log ferritin, type 2 diabetes

## Introduction

The central importance of iron in the pathophysiology of disease is derived from the ease with which iron is reversibly oxidized and reduced. This property, while essential for its metabolic functions, makes iron potentially hazardous because of its ability to participate in the generation of powerful oxidant species such as hydroxyl radical (Halliwell and Gutteridge, 1990). Evidence that systemic iron overload could contribute to abnormal glucose metabolism was first derived from the observation that the frequency of diabetes is increased in classic hereditary haemochromatosis (McClain et al., 2006). Subsequently, several other genetic disorders of iron metabolism have been reported to be associated with increased incidence of type 2 diabetes (Cario et al., 2003; Miyajima et al., 2003; Merkel et al., 1988). The association between type 2 diabetes and high body iron stores outside the setting of genetic iron overload is also recognized (Ford and Cogswell, 1999). The mechanism for the association between ferritin and type 2 diabetes is not established, but iron deposition in the liver may cause insulin

resistance by interfering with the ability of insulin to suppress hepatic glucose production (Fernández-Real et al., 2002; Moczulski et al., 2001). Iron is auto-oxidized to form highly reactive, lipid-soluble iron-oxygen complexes. These free radicals are powerful prooxidants, which can change membrane properties and result in tissue damage (Wolff, 2003; Oberley, 1998). Oxidative stress can also lead to hyperglycemia through disturbed glucose metabolism (Opara, 2004). In addition, iron accumulation in hepatocytes may interfere with the insulin-extracting capacity of the liver, and affect insulin synthesis and secretion in the pancreas (Niederau et al., 1984). Also, markers of body iron stores have been shown to be significantly higher in persons with diabetes compared with controls, even after correction for various other risk factors for diabetes including markers of obesity and inflammation (Jiang et al., 2004). Few studies have examined the relationship between serum ferritin and indices of obesity among type 2 diabetics (Shim et al., 2017; Gupta et al., 2014). Majority of such studies investigated the relationship between serum ferritin and body mass index (BMI) (Young-Eun et al., 2016). In the present study, we set out to determine the relationship between serum ferritin and BMI as well as markers of central adiposity such as waist circumference (WC), waist-to-hip ratio (WHR) and waist to height ratio (WhtR) among type 2 diabetics attending the diabetes clinic of Aminu Kano Teaching Hospital (AKTH), Kano, Northwestern Nigeria.

## Methods

In this descriptive cross-sectional study, we recruited 100 consecutive patients with type 2 diabetes attending the diabetes clinic of AKTH Kano. A pre-tested interviewer-administered questionnaire was used to obtain data on socio-demography, duration of diabetes, treatment history and history of hypertension. Patients with liver disease, sickle cell anaemia or other haemoglobinopathies, any acute illness, those with malignancy as well as patients on iron or folate containing medications were excluded from the study.

## Physical measurements

Physical measurements for weight, height, waist and hip circumferences were carried out on each patient. Weights were recorded in kilograms (to the nearest 0.5kg) using a calibrated bathroom scale (Soehnle-Waagen GmbH and Co. KG, Wilhelm-Soehnle-Strase 2, D-71540 Murrhardt/ Germany) positioned on a firm horizontal surface, with the subject clad in minimum clothing and without shoes. Height (in metres) to the nearest 0.1m was measured using stadiometer. Subjects stood erect, without shoes and headgears, on a flat with the heels and occiput in contact with the stadiometer. Body mass index (BMI) was calculated by dividing the weight (kg) by the square of the height in metres-squared (m<sup>2</sup>). Waist circumference (WC) was measured to the nearest 0.1cm (with a non-stretchable dressmaker's tape) at a point mid-way between the margin of the lowest rib and the iliac crest. Hip circumference (HC) was measured at the horizontal level of maximum circumference around the buttocks (posteriorly) and the pubic symphysis (anteriorly) to the nearest 0.1cm. Waist-to-hip ratio (WHR) was calculated by dividing the WC (cm) by the HC (cm). Waist-to-height ratio(WhtR) was calculated by dividing the WC (cm) by the height in cm. Blood pressure (BP) in millimeter of mercury (mmHg) was measured to the nearest 2 mmHg, on the right arm with subjects seated quietly after five minutes of rest with the arm and back supported using a standard mercury sphygmomanometer with an appropriate sized arm cuff. Systolic BP (SBP) was recorded at Korotkoff phase 1, while diastolic BP (DBP) was recorded at phase 5. Two readings were recorded 3 minutes apart and the average taken as the mean blood pressure. Hypertension was defined as SBP≥ 140mmHg or DBP≥ 90mmHg (Chobanian *et al.*, 2003)

# **Laboratory Methods**

Laboratory investigations carried out on each patient included serum ferritin, fasting plasma glucose, 2-hour postprandial glucose and full blood count. Ten (10) ml of blood was obtained from a prominent vein in the ante- cubital fossa using a Vacutainer needle and syringe. Five (5) ml of blood was dispensed into a bottle containing 5 mg of Ethylene diamine tetra acetic

acid (EDTA) for full blood count. Three (3) ml into a plain serum separator tube for serum ferritin and 3 ml into a fluoride/oxalate bottle for fasting plasma glucose(FPG). Participants were asked to go and eat and come back one and half hour after eating for collection of sample for 2hr post prandial plasma glucose (2HPPG). Sample for serum ferritin was centrifuged at 2000-3000 rpm after clotting for 10 min. The serum was then transferred into a serum bottle and stored at -20 °C until the time of analysis. The samples for FPG and 2HPPG were immediately centrifuged at 2000-3000 rpm and the serum frozen at -20 °C until analysis. All samples were analyzed in batches within three days of collection.

Serum ferritin was determined using a Sandwich Enzyme linked Immunosorbent Assay (ELISA) technique using a commercially available kit by WKEA Med Supplies, USA. Instruments used included; BIO-RAD Microplate washer PW 40 and BIO-RAD Microplate Absorbance Reader PR 3100 TSC. Results were calculated in ng/ml and reported in pmol/L. Glucose was analyzed by the enzymatic method of glucose oxidase (Trinder, 1969). Beckman Coulter DU 720 Spectrophotometer was used for reading absorbance. Results were calculated and reported in mmol/L. The full blood count was carried out using MINDRY BC 2400 Haematology analyzer (China). Quality of tests was assured by the use of control samples which were included in each assay run and any result that fall outside the established range was repeated. All precautions for the handling of laboratory reagents and Biosafety level 2 guidelines were observed (WHO, 2004).

## **Statistical Analysis**

The data collected were analyzed using SPSS for Windows version 16.0 (SPSS Inc. Chicago, Illinois, USA) statistical software package and STATA version 11(Stata Corporation, College Station, USA). Continuous variables were expressed as mean (SD), while categorical variables were expressed as proportions. Independent student t-test was used to compare means. Pearson's correlation coefficients were used to determine the relationship between serum ferritin and indices of obesity. A p- value of ≤0.05 was considered statistically significant.

#### Results

Of the 100 patients recruited for this study, 40(40%) were males, while 60(60%) were females. The mean (SD) age of the subjects was  $51.2 \pm 12.2$  years with a range of 25-86 years. The males were older with a mean (SD) age of  $54.9 \pm 10.0$  years compared with a mean (SD) age of  $48.6 \pm 12.9$  years for the females (p=0.01). The mean (SD) duration of DM was  $8.6 \pm 7.6$ years with a range of 1-34 years. The duration of DM was 1-5 years in 45.0% of our patients, 5-10 years in 28.0% and more than 10 years in 27.0%. There were no gender differences in the duration of DM (males -4.4 (3.8) vs. females -4.4 (3.1), p=0.53). The sociodemographic and clinical characteristics of the study subjects is shown in Table 1. Majority of our patients, 61(60.4%) were on combination therapy with Metformin and Glibenclamide, 6(5.9%) were on Pioglitazone combined with either Glibenclamide or Glimepiride, while 10(9.9%) were on a combination of Metformin and Glimepiride. The mean (SD) values of BMI, WC, WHR and WhtR of the study subjects were  $26.3 \pm 5.9 \text{ kg/m}^2$ ,  $94.5 \pm 14.9 \text{ cm}$ ,  $0.98 \pm 0.13 \text{ and}$  $0.6 \pm 0.1$  respectively. The mean (SD) BMI [27.9]  $\pm 6.2 \text{ vs. } 23.9 \pm 4.6 \text{ kg/m}^2, p=0.01$  and WhtR [0.6]  $\pm 0.1 \text{ vs. } 0.5 \pm 0.1, p=0.01$ ] were higher among females compared with males. Although the mean (SD) WC was also higher in females [96.2  $\pm$  15.9 vs. 91.9  $\pm$  12.7 cm, p=0.16], while the mean (SD) WHR was higher in males [1.01 ±  $0.14 \text{ vs. } 0.96 \pm 0.12, \text{ p=}0.11), \text{ the differences}$ were not statistically significant. The prevalence of obesity among the study subjects was 19.8%; 34(33.7%) subjects were overweight, while 8(7.9%) were underweight. Obesity was more prevalent among females than males (26.7% vs. 10.0%,  $X^2=8.59$ , p=0.03) as shown in Table 2. The mean (SD) values of SBP and DBP of the study subjects were  $131.9 \pm 22.7$  mmHg and 80.0± 12.9 mmHg respectively. Both SBP and DBP were higher in women than in men [133.9  $\pm$  23.9 vs.  $129.0 \pm 20.7$ , p=0.29 and  $81.5 \pm 13.2$  vs. 77.8 $\pm$  12.5, p=0.16 respectively,] but the differences were not statistically significant. Hypertension was detected in 27(27%) subjects, with a higher prevalence among males compared with females  $[27.5\% \text{ vs. } 26.7\%, X^2 = 0.01 \text{ p} = 0.93].$  The mean (SD) serum ferritin of the study subjects was  $79.8 \pm 88.1 \text{ ng/ml}$  with a range of 20-420 ng/ml.

The mean (SD) serum ferritin was higher among males than females [92.6  $\pm$  109.1 vs. 71.2  $\pm$  70.4, p=0.443]. Other laboratory parameters of the study subjects are shown in Table 3. The mean log transformed Ferritin (log ferritin) of the study subjects was 1.71  $\pm$  0.39. Correlation between log ferritin and the indices of obesity is shown in

Figures 2-5. Of all the indices of obesity studied, only WHR positively correlated with serum ferritin (r-0.214, p=0.48) albeit non-significantly. There was no correlation between serum ferritin and age, gender, duration of diabetes, systolic (SBP) or diastolic blood pressure (DBP) as well as FPG and 2HPPG.

Table 1: Sociodemographic and clinical characteristics of the study subjects

Variable	Male	Female	p- values
	N=40	N=60	
Age (years)	54.9(10.0)	48.6(12.9)	0.01
Duration of DM (years)	4.4(3.8)	4.4(3.1)	0.53
BMI $(Kg/m^2)$	23.9(4.6)	27.9(6.2)	0.01
WC (cm)	91.9(12.7)	96.2(15.9)	0.16
WHR	1.00(0.14)	0.96(0.12)	0.11
WhtR	0.55(0.08)	0.60(0.10)	0.01
SBP (mmHg)	129.0(20.7)	133.9(23.9)	0.29
DBP (mmHg)	77.8(12.5)	81.5(13.2)	0.16

Data expressed as means (SD). BMI=Body Mass Index. WC=Waist Circumference. WHR=Waist to Hip Ratio. WhtR=Waist to height Ratio. SBP=Systolic Blood Pressure. DBP=Diastolic Blood Pressure

Table 2: Distribution of overweight and obesity among the study subjects by gender

BMI Categories	Males	Females	Total
	n (%)	n (%)	
<18.5	6(15.0)	2(3.3)	8(8.0)
18.5-24.9	18(45.0)	20(33.3)	38(38.0)
25.0-29.9	12(30.0)	22(36.7)	32(32.0)
3360	4(10.0)	16(26.7)	20(20.0)

BMI ( $Kg/m^2$ ). BMI=Body Mass Index

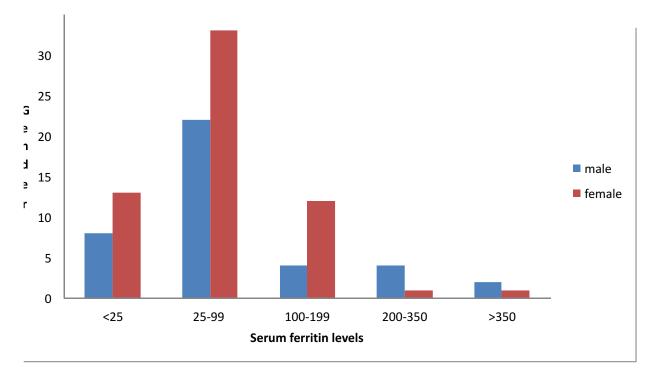
Table 3: Laboratory parameters of the study subjects

Variable	Males	Females	p-value	
PCV (%)	41.1(4.8)	39.4(3.7)	0.06	_
FPG (mmol/L)	8.7(11.8)	8.7(2.9)	0.99	
2HPPG (mmol/L)	11.9(3.9)	13.7(4.3)	0.03	
Serum Ferritin (ng/ml)	92.6(109.1)	72.2(70.4)	0.23	

Data expressed as mean (SD). PCV=Packed Cell Volume. FPG=Fasting Plasma Glucose. 2HPPG=2-Hour Post-Prandial Glucose.



Figure 1 – Distribution of serum ferritin levels by gender



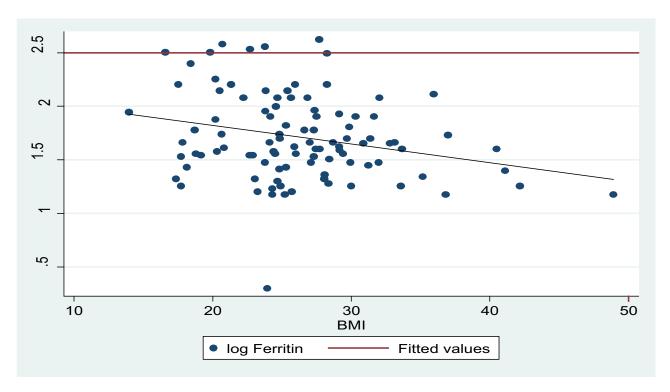


Figure 2 - Correlation between log ferritin and BMI

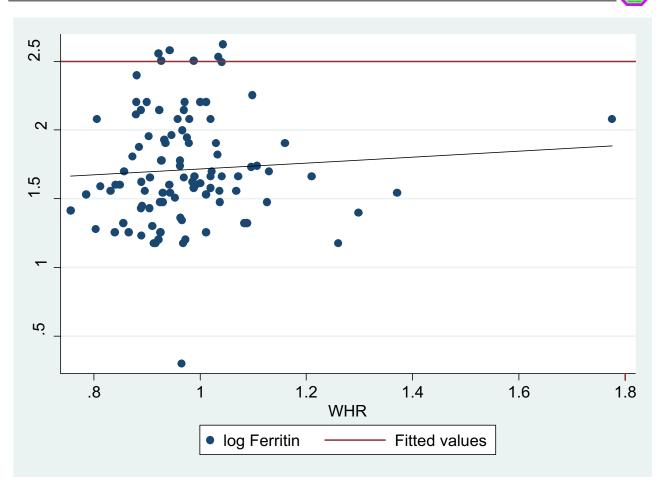


Figure 3 - Correlation between log ferritin and WHR

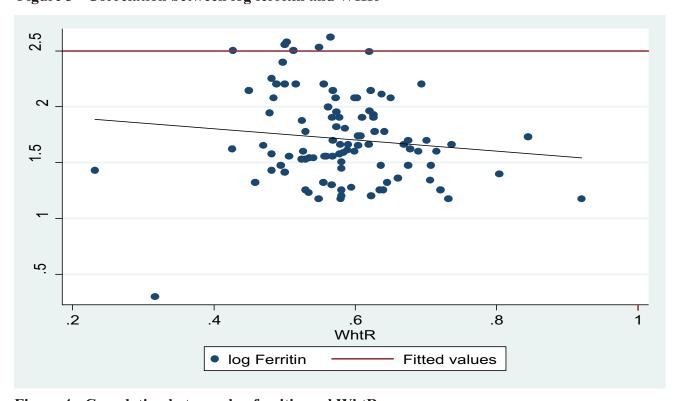


Figure 4 - Correlation between log ferritin and WhtR

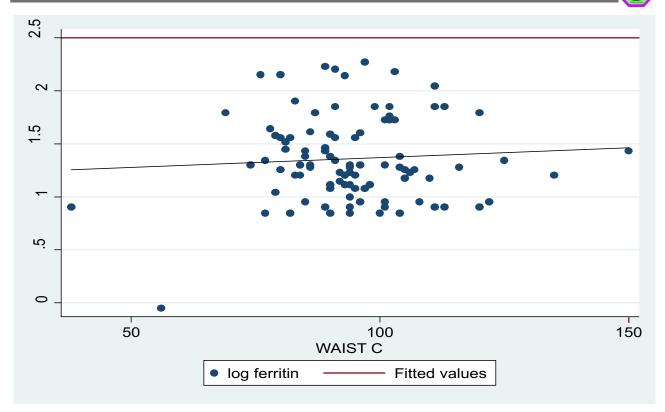


Figure 5 - Correlation between log ferritin and Waist Circumference

### **Discussion**

In this cross-sectional study, we determined the relationship between serum ferritin and indices of obesity among type 2 diabetics attending the Endocrine and Diabetes clinic of our Hospital. There were more females than males in our study sample, a finding that is similar to the observation by Ogbera et al., (2010) in Lagos, Southwest Nigeria, but in contrast with the report by Isezuo and Ezunu, (2005) from Sokoto in the Northwestern part of the country. The female preponderance among our subjects may be related to the fact that more women seek medical attention than men in our setting. Other studies have reported higher proportion of males than females among their subjects (Ogbera et al., 2010; Alebiosu and Odusan, 2004). The mean age of our study subjects was  $51.2 \pm 12.2$  years, similar to the findings of Isezuo and Ezunu, (2005) in Sokoto. In this study, we found the prevalence of obesity to be higher among females than males. Edo and Edo (2012) from Benin Midwestern Nigeria and Olube et al., (2014) from Enugu Southeastern Nigeria have reported similar findings. Cultural practices that tend to limit physical activity in females as well as hormonal changes are thought to be responsible for the gender-related differences in obesity rates. Majority of our study subjects had serum ferritin levels in the lower limit of the normal range. Pramiladevi et al. (2013) reported similar findings among their patients with type 2 diabetes. Neither age nor gender were found to correlate with log ferritin in this study. This concurs with the observations of Raj and Rajan (2013). These authors also reported a positive correlation between serum ferritin and duration of diabetes among their subjects with majority of them having had diabetes for more than 10 years (Raj and Rajan, 2013). In contrast, our study failed to establish any relationship between log ferritin and duration of diabetes. The fact that majority of our subjects had diabetes for less than 10 years may explain the difference observed. Of the indices of obesity assessed in this study, only WHR showed positive correlation with log ferritin. This finding is novel in that most studies on the relationship between WHR and serum ferritin involved non-diabetic subjects (Aderibigbe et al., 2011; Gillum, 2001). WHR is an index of central adiposity. The mean WHR of subjects in this study was high reflecting high rates of central obesity, an important component of the insulin resistance syndrome. In several

studies, serum ferritin was correlated with components of the insulin resistance syndrome (Fernandez-Real et al., 1998; Berge et al., 1994). We found a negative and significant correlation between BMI and log ferritin. Some studies have reported positive correlation between log ferritin and BMI (Aderibigbe et al., 2011; Gillum, 2001). Other workers however, found no relationship between log ferritin and BMI among their subjects (Fernandez-Real et al., 1998; Berge et al., 1994). In this study, both FPG and 2HPPG did not correlate with log ferritin. This contrasts with the observations of Chandrashekhar et al. (2014) as well as with the report by Okonkwo and Okoye (2014) who found positive and significant correlation between serum ferritin and FPG among their subjects with type 2 diabetes. Both systolic and diastolic blood pressures did not correlate with log ferritin in our study. Raj and Rajan (2013) reported similar findings. Other studies have however reported positive and significant correlations between serum ferritin, systolic and diastolic BP as well as coexistent hypertension among their subjects (Okonkwo and Okoye, 2014; Pramiladevi et al., 2013; Smotra and Kudyar, 2008). The lower prevalence of hypertension among our subjects compared with the latter studies may explain the observed difference. A previous Nigerian multicenter study by Uloko et al., (2012) however reported a higher prevalence of systemic hypertension (over 60%) among persons with diabetes mellitus.

The limitations of our study include its crosssectional design and the relatively small sample size employed which makes generalization of the findings difficult. Although efforts were made to exclude patients with any acute illnesses, patients with subclinical conditions may have been missed. Since serum ferritin is an acute phase reactant its levels may be raised in such conditions potentially affecting the results from this study. The fact that we found positive correlation between WHR, a marker of central adiposity, and serum ferritin in this study infers that the latter among our subjects is related to components of the insulin resistance syndrome. We did not however assess insulin resistance in this study which would have shed more light on the relationship between serum ferritin and the syndrome.

### Conclusion

Our study revealed that serum ferritin levels were in the lower limit of normal in the majority of our patients. There was no correlation between age, gender, duration of DM, FBS and 2HPPG and serum ferritin among our subjects. The overall prevalence of obesity in our study sample was high, but in tandem with reports from similar studies. Of the indices of obesity only WHR correlated positively with serum ferritin. There is need for further studies employing a larger sample size to establish the relationship between serum ferritin and variables such as HbA1C and insulin resistance (IR) in addition to indices of obesity in our patients with type 2 diabetes.

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### **Authors Contribution**

All authors contributed significantly to the writing of the manuscript. All authors read and approved the final manuscript.

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