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Relationship between higher Serum ferritin levels, Insulin resistance marker and components of metabolic syndrome in Men and Women in west part of India

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Abstract

Background: There are no effective studies in west part of India on relationship between higher serum ferritin levels in impaired glucose individuals, type 2 diabetes mellitus patients are markers for insulin resistance and reinforce to metabolic syndrome. The association between serum ferritin levels, insulin resistance marker (HOMA IR), Glycosylated Hemoglobin A1C with individual components of metabolic syndrome in type 2 diabetes mellitus, impaired fasting glucose, obesity and healthy subjects are unclear.
Objectives: This research was delineated to find out the interrelation between serum ferritin levels with fasting glucose levels, waist hip ratio, fasting insulin levels, insulin resistance marker (HOMA-IR) and components of metabolic syndrome in type 2 diabetes mellitus patients, newly identified type 2 diabetes mellitus patients, Impaired fasting glucose subjects and healthy participants. To investigate any menopause transition effects on serum ferritin levels and components of metabolic syndrome for identifying insulin resistance and early diagnosis of type 2 diabetes mellitus in men and women of age 30-65 years.
Subjects and Methods: The number of individuals included in this study 1058, out of them 365 patients with previously diagnosed type 2 diabetes mellitus with poor and good glycemic control patients, 144 patients with newly identified type 2 diabetes mellitus, 189 with participants with impaired fasting glucose levels and 360 healthy participants. Ferritin levels in serum, fasting glucose in plasma, fasting insulin levels in serum, Glycosyalted HbA1c in blood and serum lipid levels were analyzed. Waist Hip ratio is measured. Homeostasis Model Assessment-Insulin Resistance (HOMA-IR) is calculated.
Results: In our analysis, out of 1058 participants, 289 individuals (27.2%) with increased levels of ferritin levels in which 46.6% (newly identified type 2 diabetes mellitus patients), 31.95% previously diagnosed type 2 diabetes mellitus 20.4% impaired fasting glucose subjects and 10.55% healthy participants. The odd ratios for men in newly diagnosed diabetes mellitus were 4.94 and for women 3.61. Linear regression analysis between serum ferritin, blood insulin, and glucose and glycosylated hemoglobin showed significant correlation.
Conclusion: our research findings suggest increased ferritin in blood as marker for insulin resistance and early indicator for diagnosis of type 2 diabetes. High ferritin in metabolic syndrome identify early indicator for insulin resistance and pre diabetes mellitus.

Keywords: Mets; GHB; HOMA-IR.

Introduction

India is cardinal in world for diabetic patients and termed the "capital of diabetes in the world". The roughly estimated prevalence of type 2 diabetes mellitus will be twofold increased in India by the year between 2000 and 2030.[(1)] International Diabetes Federation (IDF) confirmed roughly estimated prevalence of type 2 diabetes patients in India were 7.8% in 2010 for approximately 51 million people, considering World Health Organization Criteria the earlier study in urban India showed greater prevalence 12.1 %.[(2)]

International Diabetes Federation and World Health Organization strongly anticipated that type 2 diabetes will be increased up to 9.3% by year 2030 with percentage of Indians; the explanation may be due to migration of humans from rural to urban areas for employment, change in dietary habits and shift to steadily more sedentary life style.[(3,4)]

In study done by American pathologist in type 2 diabetes mellitus patients along with hemochromatosis showed rise serum ferritin levels.[(5)] However, it is foggy whether fairly elevated serum ferritin levels speculate the probability of progressing to type 2 diabetes mellitus and its complications among healthy subjects. Unstable free radicals generated by free iron causing Biological Membrane to get lysis, alters protein structural configuration and dislodge nitrogen bases in gene sequences.[(6,7)]

Previous research gives about comprehensive hypothesis on free iron to hydroxyradicals which damages pancreas, contributes primarily to insulin resistance and then progress to type 2 diabetes mellitus.[(8,9)] Several cross sectional and case control studies manifest positive association between high serum ferritin levels and insulin resistance progress to type 2 diabetes mellitus and its complications.[(10-13)]

High serum ferritin concentrations may exhibit systemic inflammation which predicts insulin resistance rather than increased serum ferritin levels because blood samples are collected after the diagnosis of diabetes mellitus. Retrospective or cross-sectional data cannot create directionality association between insulin resistance and serum ferritin levels.[(14)]

The impact of free iron on type 2 diabetes mellitus and metabolic syndrome had reveal with the emerging scientific evidences; also higher glucose levels effects on

metabolic pathways of iron which are bi-directional. Chronic diabetes complications are regulated by free iron induced damage. In large clinical trials, type 2 diabetes mellitus patients after treatment showed reduced free iron levels.[15]

We had conducted this large cross sectional study to find out the hypothesis in which higher ferritin levels may impairs glucose utilization by insulin resistance and progress to type 2 diabetes mellitus.

Study design

Participant enrollment: This study initiated and started after Approval by Research Advisory Committee (RAC) and SBKS MI and RI Ethics committee**.** The need and benefits of the study had been explained to willing participants in their own language and written informed consent taken. All participants in this study asked to report, age, sex, alcohol intake, smoking, how often, on average, during the previous year they had consumed selected foods, beverages, and number of blood donations done, information recorded case record form. Habitual alcohol consumption was inquired with the following two questions. Do you drink at least once a month? Yes/no.

Study population: The study conducted on participants and patients of age ≥30-65 years old men and women who visit to Dhiraj Hospital (DH) a territory Care 1226+Bedded Teaching hospital located in Pipariya, Vadodara to check for Diabetic Status and routine checkup. Pregnant women's participants who fasted less than 8 hrs at the time of examination excluded from this study.

Study period: August-2011 to September Hospital based cross sectional study**:** According to new diagnostic criteria**,** Participants in this study are categorized into four groups**.** These are Group 1 (Previously diagnosed case of type-2 DM), Group 2 (Newly Diagnosed type 2 DM patients, fasting glucose concentration of >7.0 mmol/l (>126 mg/dl),), Group 3 (Impaired fasting glucose subjects a fasting glucose concentration of 5.6 to 7.0 mmol/l (100-125 mg/dl)) and Group 4 (Healthy controls subjects fasting blood glucose concentration <6.1 mmol/l (<110 mg/dl).

Based on the glycemic level (HbA1c), (previously diagnosed cases of diabetes mellitus patients were sub categorized into type 2 Diabetes mellitus with poor glycemic control HbA1c>6.5% and type 2 Diabetics mellitus with good glycemic control HbA1c <6.5%.

Physical examination: Body Mass Index of Participants, calculated using measured weight in kilograms divided by the square of height of height in meters. Waist circumference was measured by a trained technologist to the nearest 0.1 cm in the horizontal plane at the level of the high point of the iliac crest at minimal respiration. Systolic and diastolic blood pressure (BP Systolic and BP Diastolic) were measured using a BP apparatus name sphygmomanometer by physician in Medicine OPD and Diabetic Clinic noted in CRF form

Biochemical Methods and Blood collection: Physician examines participants and patients enrolled in this study from Department of Medicine and Diabetic clinic OPD. Blood samples collected from all participants and patients at Central Laboratory, Dhiraj Hospital, samples collection centre. Minimum 8-10 ml of venous blood collected from each participant after an overnight fasting i.e. at least 8 hr fasted without calorie intake (2ml fluoride oxalate vaccutes for glucose estimation, 2 ml into EDTA vaccutes for some haemotological parameters like Hb% performed immediately and remaining investigation lipid profile, C-reactive protein, Fasting Blood insulin in plain vaccutes. Plain samples for analysis allowed to clot for 30 minutes and centrifuged 3000 rpm for 15 minutes for fair separation of serum.

Glucose: Fasting blood glucose by glucose oxidase and peroxidase (GOD POD) analysed on auto-analyzer-ERBA-EM-200 Trans Asia.[16]

Serum ferritin: Samples with ferritin concentration agglutinated with anti-ferritin antibody coated on latex particles**.** Change in absorbance due to agglutination in Nephlometry dependent on the ferritin in the sample**.** Instrument and reagents are calibrated with the calibrators.[17,18]

Glycosylated hemoglobin (HbA1c): Whole blood samples collected in EDTA tubes analysed by using method Immuno assay nephlometry method in which antihuman HbA1c monoclonal antibodies is added (R2), HbA1c-mouse antihuman HbA1C antibody complex formed. Goat anti-mouse IgG polyclonal antibody interacts with the monoclonal antibody leads to agglutination. The amount of agglutination is proportional to the amount of HbA1c absorbed on the surface of latex particles. The amount of agglutination is measured to calculate HbA1c from calibration curve.[19]

Lipid profile:

Total cholesterol: Serum total cholesterol estimated by CHOD-PAP Method on automated analyzer- EM 200.[20, 21]

Triglycerides: Serum Triglycerides estimated by Glycerol phosphate oxidase (GPO) Method on automated analyzer EM 200.[22-24]

HDL-Direct: HDL- Direct Cholesterol estimated by Immuno Inhibition in which enzymees selectively react with HDL to produce H202 which is detected through a trinder reaction.[25,26]

Insulin ST AIA Pack IRI: Samples of Fasting blood Insulin levels estimated by two-site Immuno-Fluro-enymometric Assay on Automated Tosoh AIA 360.

Iron (CAB): Iron in the sample reacts with cetyltrimethyl ammonium bromide (CTMA) to form a coloured ternary complex which is observed at absorbance 623 nm**.**[27]

Total Iron Binding Capacity (TIBC): serum transferrin which is called total iron-binding protein is saturated up on treatment with an excess of Fe (III) ions,

unbound excess iron is adsorbed on to aluminum oxide and precipitated. The transferring-bound iron (TIBC) in the supernatant is then used.[28]

Hemoglobin (Hb): Total Hemoglobin levels in blood estimated by the cyanide sodium lauryl sulphate method.[29]

Metabolic syndrome: Participants with following at least three investigations: elevated Blood pressure, low HDL Cholesterol, elevated triglycerides, elevated blood glucose, and central obesity measurement were included under metabolic syndrome and components of metabolic syndrome according to each investigation, the metabolic syndrome was defined by the national cholesterol Education program Adult Treatment Panel III guidelines.[30]

HOMA-IR: Homeostatic model assessment is a method for assessing β-cell function and insulin resistance from basal fasting glucose and insulin levels in blood. The formula of homeostasis model assessment for insulin resistance (HOMA-IR) Fasting insulin in blood (Uiu/ml) x fasting glucose (mg/dl)/405, even though this parameter is not a gold standard method for insulin resistance, many studies proved it good parameter to correlates insulin resistance. The participants who had HOMA-IR score more than 2.5 were categorized under insulin resistance. The other way to find out insulin resistance is by triglyceride to HDL Cholesterol ratio.[31]

Statistical analysis: we used SPSS software version 16.0. Data were expressed in terms of mean±SEM. Analysis of participants data determined statistically significant or not by student's t-test. Statistically significance is considered when probability value < 0.05. Linear regression analysis done on serum ferritin, fasting glucose, and fasting insulin to find out contrast correlation between investigations and insulin resistance.

Results and Discussion

In our analysis, out of 1058 participants, 289 individuals (27.2%) with increased levels of ferritin levels in which 46.6% (newly identified type 2 diabetes mellitus patients), 31.95% previously diagnosed type 2 diabetes mellitus 20.4% impaired fasting glucose subjects and 10.55 % healthy participants shown in Table 1.

Table 1: Distributional information of serum ferritin concentration by selected variables among participants aged ≥30-65years

Characteristic	Sample size (n)	Geometric mean ferritin (ng/ml)	Percentage with elevated ferritin concentration
Total	1058	225.25±12.8(2-1,979)	27.285±2.91
Men	696	244.33 ± 18.5(3-1.603)	26.925±3.35
Women	362	198.75± 14.6 (2-1.979)	27.65±2.475
Diabetes status Normal			
Men	222	118 ±6.2 (3-1.698)	11.4±1.1
Women	138	98 ±4.2 (3-1.404)	09.7±0.9
Impaired fasting glucose subjects			
Men	108	216.3 ±11.9(3-1.742)	18.2±2.4
women	81	145.3 ±9.7(3-1.143)	22.6±1.7
Newly diagnosed diabetes			
Men	92	320.3 ±17.9(18-1.137)	44.9 ±5.1
women	52	230.2 ±14.2 (3-729)	48.3 ±4.2
Previously diagnosed diabetes Men			
women	246	278.05±17.3(3-1.354)	33.2 +4.8
	119	187.5±13.1(3-1.070)	30.7 +3.1

In Poor glycemic control patients (HbA1c>6.5%) the mean serum ferritin levels are higher compared with good glycemic control patients (HbA1c<6.5%), along with components of metabolic syndrome, HOMA-IR, and duration of type-2 diabetes in previously diagnosed type-2diabetes mellitus shown in Table 2.

Table 2: Distributions of mean serum ferritin, components of metabolic syndrome, HOMA-IR, and duration of type-2 diabetes in previously diagnosed type-2diabetes mellitus with poor glycemic control (HbA1c>6.5) and good glycemic control (HbA1c<6.5)

	Group-1a (HbA1c>6.5) (poor glycemic control)	Group-1b (HbA1c<6.5) (good glycemic control)	P analysis
n	204	161	-
Sex M/F	145/65	101/54	-
Age	57.06±7.9	55±7.2	-
Duration	5±3.6	4.5±2.5	-
BMI kg/m^2	32.72±4.86	24.58±2.65	<0.001
Alcohol use(drinks/month)	3.5±1.0	3.0±0.8	0.007
FBG (mg/dl) *	175±14	110±10	<0.001
Mean serum ferritin in ng/ml	308±27	233±17	<0.001
Serum C-reactive protein(mg/l)	5.9±1.4	2.9±0.9	<0.001
Systolic blood pressure(mm Hg) ł	132.50+11.0	129.6+10.1	<0.001
Waist hip ratio	1.074±0.04	0.9935±0.04	<0.001
T. cholesterol in mg/dl.	243.85±32.44	194.65±23.44	<0.001
Triglycerides in mg/dl	194.65±22.40	158±18.60	<0.001
HDL. cholesterol in mg/dl	36.40±7.1	42.54±6.4	<0.001
Fasting insulin in Uiu/ml.	9.60 ±2.45	7.88±1.90	<0.001
HOMA-IR	4.10+1.65	2.10+0.90	<0.001

Findings in present study agree with a previous prospective cohort study done on 944 subjects, in which base line ferritin and transferring levels correlated with fasting glucose and HOMA-IR.[32] Insulin action is defective in insulin resistance. In a Broad clinical spectrum of insulin resistance; varying from euglycemia (with marked increase endogenous insulin levels) to hyperglycemia. We should note from previous studies done on rats, insulin responsible for increased ferritin synthesis in cultured glioma cells.[33]

Persistant increased serum ferritin levels in blood may lead to free iron release and altered glucose homeostasis in two different ways. First hypothetically high serum ferritin levels in blood leads deposition in the pancreas which impairs synthesis and secretion.[34] Secondly, accumulated ferritin could interfere with the insulin-extracting capacity of the liver resulting in hyperinsulinemia.[35] Liver with accumulated Iron may cause insulin resistance by interfering insulin action to utilize glucose by hepatic cells.

In our research findings both fasting blood glucose, blood insulin levels are elevated along with the serum ferritin concentrations. Previous research findings recommend that in inclusion to the glucose elevation, serum ferritin levels befit a surrogate marker for early diagnosis of type 2 diabetes mellitus.[36]

In our analysis the mean serum ferritin levels found lower in premenopausal women (46.5 ng/ml 95% CI) contrast with postmenopausal women 118.36 ng/ml and men 187.50 ng/ml (shown in Table 3 and Fig. 1). Segregated menopause status from pre to post menopause women, consistent high ferritin levels and diminished ratio of serum transferring ratio (sTfR) ferritin levels. In menopausal transition we recorded major participants with insulin resistance and hyperglycemia. Women in menopausal transition had tremendous modifications in their blood ferritin and iron levels (i.e. in premenopausal with lesser ferritin, iron and postmenopausal with higher levels of ferritin, iron) evinced the valid correlation between serum ferritin and HOMA-IR. Our research findings also recommends pathway through which women undergoing the menopausal transition may endangers to insulin resistance, in support to study conducted on menopause transition with unfavorable life style changes,[37] and increasing ratio of androgen: estrogen characterizing menopause.[38]

Table 3: Geometric mean values of serum ferritin are shown for premenopausal, postmenopausal women and men

Components of metabolic syndrome	Premenopausal women Mean serum ferritin ng/ml	Postmenopausal women Mean serum ferritin ng/ml	Men Mean serum ferritin ng/ml
0	34.6	67.2	132
1	39.8	82.4	139.8
2	44.3	92.5	166.3
3	54.2	151.2	230.6
>4	59.6	198.6	268.9

Total Mean ferritin	46.5	118.38	187.52

In our study, serum ferritin levels strongly correlated with mean systolic blood pressure (135.5 mm of Hg), and mean fasting insulin (10.93 uIU/ml) in adult metabolic syndrome patients (shown in Table 4), this evidence supported by study conducted on postmenopausal women with elevated serum ferritin (200 ug/L) manifested 3.77 fold higher risk of stroke contrast with normotensive women.[39] In hypertension hypothetically due to generation of superoxide radicals which leads to impaired endothelium-dependent vaso-relaxation. In several cross sectional studies the each cardiovascular risk investigation including hypertension and central obesity strongly correlated with serum ferritin levels.[40,41]

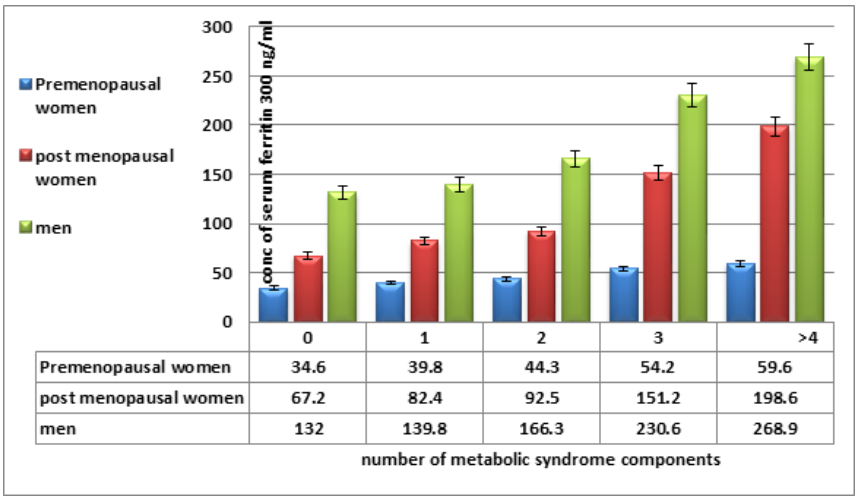

Fig 1: Distribution of mean serum ferritin levels in components of metabolic syndrome

In our study even though the mean values are less but there is significant effect of alcohol on serum ferritin (P<0.001) in previously diagnosed type 2 diabetes (Group 1), Newly diagnosed type 2 diabetes mellitus (Group 2), Impaired fasting glucose (Group 3) compared to healthy participants (shown in Table 4). The odd ratios related to elevated ferritin with normal glucose levels in newly diagnosed type 2 diabetes found 4.94 (95% CI. CI 3.05-8.01) in men and women 3.61(2.01-6.48).

Table 4: unadjusted mean of serum ferritin, metabolic syndrome components and insulin resistance syndrome (HOMA-IR) among the participants aged ≥30 -65 years

	Healthy subjects* (Group 4)	**Impaired fasting glucose (Group 3)**	**Newly diagnosed diabetes (Group 2)**	**Previously diagnosed diabetes (Group 1)**	***P*(analysis)**
Age (years)	46.2 (1.0)	56.13 (0.9)†	59.0(1.0) †	57.9 (0.8) †	<0.001
Sex (M/F)	222/138	108/81	92/52	246/119	
Alcohol use (drinks/month) %	2.3 (0.3)	2.0(0.25) †	2.0(0.72) †	2.2(0.8) †	0.008
Smoker /day	1.8±0.4	2.0±0.50	2.6±0.9	2.3±0.7	<0.001
BMI (kg/m2)	20.66±2.13	23.83 ±2.71	33.53 ±4.66	30.26 ±3.48	<0.001
Systolic blood pressure (mmHg)	117.5(0.4)	132.8(1.1) †	135.5 (1.5) †	131.7 (1.8) †	<0.001
Fasting blood glucose (mg/dl)	84±6.3	119±12.0	165.6±16.4	142.5±14.7	<0.001
serum ferritin (ng/ml)	94.66±2	207.6 (0.04) †	325(0.02) †	270.33 (0.01) †	<0.001
Serum insulin uU/ml	5.8± 0.88	7.55 ±1.07	10.93±2.93	8.81 ±2.46	<0.001
Total cholesterol (mg/dl)	140.50 ± 15.12	182.6 ±22.56	238.3±30.7	219.40 ±29.21	<0.001

HDL cholesterol (mg/dl)	51.08 ± 4.2	45.38 ± 5.8(0.02) †	38.2±8.86 (0.03) †	39.47±6.75(0.03) †	<0.001
Triglycerides (mg/dl)	116±15	143.6 ±16.8	188±18.3	156.33 ±20.23	<0.001
HOMA -IR	1.26 (0.79 -1.75)	2.22 (2.9-3.95)	4.4 (2.4-6.4)	3.15 (2.07 -4.07)	<0.001
HbA1C	5.2±0.2	5.9±0.4	12.8 ±1.2	8.93 ±0.75	<0.001
Transferrin saturation %	28.9(0.3)	26.7(0.5) †	24.5 (0.8)	26.23 (0.8)	<0.001
TGL/HDL-C ratio	2.156±0.4	3.18±0.6	4.94±1.2	3.96±0.8	<0.001

Table 5: Odd ratio for four categories in newly diagnosed type-2 diabetes mellitus patients

Transferrin saturation <45% <300 ng/ml men <150 ng/ml women	1.0
Transferrin saturation <45% >300 ng/ml men >150 ng/ml women	4.22(2.97-5.99)
Transferrin saturation >45% >300 ng/ml men >150 ng/ml women	0.30(0.09-1.02)
Transferrin saturation >45% <300 ng/ml men <150 ng/ml women	1.79(0.60-5.33)

Conclusion

Our findings concludes that significant increased ferritin levels in blood with adjusted CRP, WBC cells in participants with all components of metabolic syndrome manifest certain degree of inflammation which tends to decrease insulin action by rising HOMA-IR Score which is early indicator for development of type 2 diabetes and cardiovascular risk factors. The participants with elevated ferritin levels and Hb> 11.5 % along with the impaired glucose levels recommended to set routine investigation in chemical pathology lab for diagnosis of insulin resistance and metabolic syndrome.

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