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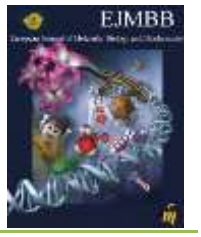

AN ELEVATED FERRITIN LEVEL IS AN INDICATOR TO IDENTIFY METABOLIC SYNDROME AND INSULIN RESISTANCE IN IMPAIRED FASTING GLUCOSE SUBJECTS

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ABSTRACT

The main aim of this study to find out elevated serum ferritin relates the risk of metabolic syndrome and insulin resistance in healthy participants and impaired fasting glucose respondents. Methods: In this study the participants are categorized in to two groups based up on the fasting blood glucose levels. Group 1 healthy participant those are visiting from factories, police, and army camps to Dhiraj Hospital as part of company health scheme for routine checkup and found to have no disease or fasting glucose levels below 100 mg/dl considered as healthy or control subjects. Group 2 impaired fasting glucose respondents whose fasting blood glucose lies between 100-125 mg/dl (5.6-7.00 mmol/l). This study included 219 respondents with impaired fasting glucose levels and 378 healthy participants. Total number of participants included 607 from period August 2011 to March 2015. Serum ferritin levels, fasting plasma glucose, serum insulins levels, glycosylated hemoglobin levels, fasting lipid profile levels, components of metabolic syndrome (measurement of BMI, waist: hip circumference ratio, measuring blood pressure and estimating blood triglyceride, HDL-Cholesterol levels) and insulin resistance markers homeostasis Model Assessment-insulin resistance (HOMA-IR), were calculated by measuring fasting insulin and glucose levels. The present study was restricted to participant's women who were not pregnant at the time of examination. In this study participants who fasted <8 hrs at the time of examination are excluded. Results: The more number of respondents with elevated ferritin in impaired fasting glucose group (men: 24.21%; women: 27.57%). The odd ratio for men 5.59 (95% CI: 2.8519-10.968) and women 4.88(95% CI: 2.158-11.0747) in percentage of individuals with elevated ferritin in impaired fasting glucose respondents. Conclusion: This study reveals an elevated serum ferritin levels indicates more chances for developing insulin resistance syndrome and metabolic syndrome in adults..

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INTRODUCTION

In world wide the prevalence of metabolic syndrome is 20-25% in adult population. Elevated levels of 3 or more than 3 components of metabolic syndrome and insulin resistance results in contributing cardiovascular risk factor and type 2 diabetes mellitus respectively in adults[1]. Ferritin (iron stores) is a ubiquitous intracellular protein which play key role in regulation of iron homeostasis [2]. Function of ferritin is an inherent feature of the H subunit which has feeoxidase activity. Small quantity of ferritin is also present in human serum, is elevated in conditions of iron overload, and inflammation. Serum ferritin is iron-poor, resembles ferritin L

immunologically, and many contain a novel "G" (Glycosylated) subunit [3] as shown in figure 1. The free iron donates unpaired electrons for the generation of superoxide's a free radical which is resulted from generation of reactive species. The hydroxyl radicals generated from free iron via Fenton's reaction [4] causes damage to DNA, Lipids and Proteins eventually to cellular toxicity. This is the pathological basis to develop diabetes mellitus, neoplasia and degenerative brain disorders [5, 6]. However, strong evidence from met analysis signifies that elevated ferritin levels relate to development of type 2 diabetes mellitus [7-9]. Further, Study conducted in comparison with poor and good glycemic control patients based up on HbA1c levels demonstrates elevated ferritin levels in poor glycemic control subjects [10]. Impaired fasting glucose is a stage of impaired glucose homeostasis in which range of fasting glucose was revised and standardized to 100-125 mg/dl (5.6-<7.0 mmol/l)[11]. Recent studies demonstrates elevated serum ferritin significantly high in newly diagnosed type 2 diabetes mellitus, previously diagnosed type 2 diabetes and impaired fasting glucose respondents in comparison with healthy participants [10, 19]. In detail, it is not clearly explained about moderately elevated serum ferritin levels for long time causes insulin resistance and metabolic syndrome in adults. This study gives information about even slightly elevated serum ferritin levels effects on various components of metabolic syndrome and investigation which used to assess insulin resistance syndrome.

Metabolic syndrome:

The metabolic syndrome was defined based on the national cholesterol education program Adult Treatment Panel III criteria for Asia Pacific [13,14]. Respondents with presence of $\geq$3 criteria listed below were classified with metabolic syndrome MetS [13]. 1. Overweight as BMI>24 Kg/m2 and obese as BMI>27 Kg/m2: 2) waist circumference of >90 cm in men and >80 cm in women: 3) TG>150 mg/dl: HDL-C<40 mg/dl for men and <50 mg/dl for women: 5) systolic B.P>130 mmHg or Diastolic BP>85 mmHg or current use of antihypertensive drugs: and 6) fasting blood glucose >100 mg/dl or current use of anti-hyperglycemic drugs.

Insulin resistance

Insulin resistance is defined as declined biological function of insulin even though a sufficient concentration of endogenous circulating insulin is presented [15]. Insulin resistance is usually attributed to a defect in insulin action. For respondents with normal glucose levels, serum insulin is a good index of the severity of insulin resistance, because the ability of the beta-cell to produce insulin together with magnitude of the insulin resistance, will determine the overall degree of hyperinsulinemia. Though, the gold standard method for measurement of insulin sensitivity is glucose clamp test [16], Homeostasis model assessment (HOMA-IR) is a method used widely to ensure insulin resistance and evaluate insulin sensitivity in patients with type 2 daibetes mellitus [17].

MATERIALS METHODS

This study initiated and started after approval by research advisory committee and institutional ethics committee. The participants willing to participate in this study have been explained about the purpose and method of study and method of the study, in their own language and their written informed consent taken. The Gujarati consent form enclosed. All participants informed to report age, sex, alcohol intake, smoking, how often on average during previous year they consumed food, beverages and number of blood donations done, information attached case record form (CRF) annexure.

Statistics

Statistics used with the help of SPSS software version 16.0. Data taken from this study analyzed to get mean+ SD. Mean values are compared with standard deviation. Linear regression, Pearson correlation coefficient and student's t-test were performed to find significance levels in comparison of two groups. Odd ratio is calculated for men and women in comparison between impaired fasting glucose respondents and healthy particiapnts to find whether exposure is positively associated with disease. To find out ferritin as the dependent variable multiple regression analyses were performed.

Study population

Total number participant's included 607 from period August 2011 to March 2015.This study included 219 respondents with impaired fasting glucose levels whose strictly follows fasting and estimated blood glucose levels founds between 100-125 mg/dl tested clinical Biochemistry laboratory, Dhiraj hospital and 378 healthy participants those are working from factories, police, and Army camps Visit to Dhiraj Hospital as part of company health scheme for routine checkup considered as healthy or control subjects.

Study period

From August 2011 to March 2015

STUDY DESIGN

Hospital based cross sectional study:

In this study the participants are categorized in to two groups based up on the fasting blood glucose levels. Group 1 healthy participant's those are visiting from factories, police, and army camps to Dhiraj Hospitial as part of company health scheme for routine check up and found to have no disease or fasting glucose levels below 100 mg/dl considered as healthy or control subjects. Group 2 impaired fasting glucose respondents whose fasting blood glucose lies between 100-125 mg/dl (5.6-7.00 mmol/l). The study participants are strictly monitored

whether they follows 8hr fasting as per American diabetes association (ADA) criteria [11]. Errors in clinical laboratory mainly affect the results of the study and clinical decisions of physician or surgeon. Therefore, Errors in clinical laboratory are identified and reduced by application FMEA and by following structured approach as proven in previous studies [12, 18]. We calculated mean BMI of respondents with impaired fasting glucose. Participants with recent blood transfusions and pregnant women are excluded from this study. We also excluded participants with chronic infections are excluded by analyzing C-reactive protein.

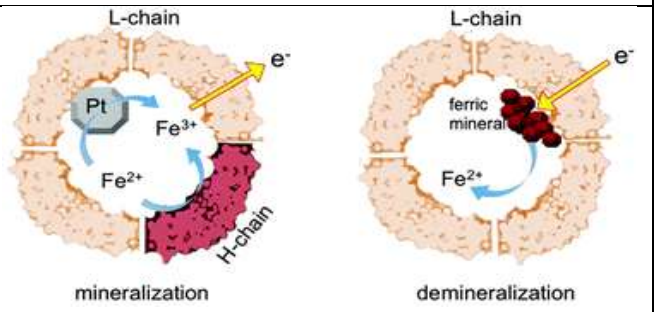
Sample collection

All participants and patient samples collected at sample collection centre, Dhiraj hospital, Pipariya, vadodara. Participants after physical examination done by Physician in Department of Medicine or Diabetic clinic are prescribed for blood analysis. Minimum 8-10ml of venous blood was collected from each volunteer after an overnight fast minimum 8hrs without calorie intake. 2 ml of blood transferred into fluoride oxalate vaccutainer for analysis of glucose. 3ml of blood transferred into EDTA vaccutes for hematological and HbA1c analysis. 3 ml of blood transferred in to plain vaccutes for analysis of lipid profile, C-reactive protein, fasting insulin levels. Plain vaccutes allowed to clot for 30 minutes and centrifuged 3000 rpm for 15 minutes for clear separation of serum.

RESULTS

After following exclusion criteria total 607 adult participants included in this study. In this study the participants are categorized in to two groups based up on the fasting blood glucose levels. Group 1 healthy participant those are visiting from factories, police, and army camps to Dhiraj Hospitial as part of company health scheme for routine check up and found to have no disease or fasting glucose levels below 100 mg/dl considered as healthy or control subjects. Group 2 impaired fasting glucose respondents whose fasting blood glucose lies between 100-125 mg/dl (5.6-7.00 mmol/l).This study shows mean serum ferritin levels (180.80± 19.4ng/ml), fasting blood of glucose levels (119±12 mg/dl) and Components of metabolic syndrome (Systolic blood pressure:132.8±9.2, Total cholesterol 182.2±22.56, HDL-Cholesterol 45.2 ± 5.8, triglyceride levels 143.5±12.4, BMI 23.96 ±2.63, waist hip ratio 0.928±0.04 and insulin resistance calculation marker (HOMA-IR 2.21±0.3) in impaired fasting glucose subjects (N=219) were found higher compared with healthy control subjects(Group 1) (N=378) P<0.001). The more number of respondents with elevated ferritin in impaired fasting glucose respondents (men: 24.21%; women: 27.57%) as depicted in figure 1 and 2. The odd ratio shows very significant positive correlation with risk of metabolic syndrome in comparison of healthy group as shown table 7 (men=6.02: women 5.30) (r=+1 table 4&5).

Figure 1: **shows mineralized and de mineralized form of ferritin**



Courtesy: Royal society of chemistry

DISCUSSIONS

Our study finding demonstrates elevated serum ferritin levels with impaired fasting glucose levels suggest risk of developing insulin resistance syndrome and effects on various components of metabolic syndrome to be elevated which eventually leads to cardiovascular disease which significantly correlates with results of previous study [20]. In this study the impaired fasting glucose subject the waist circumference mean values are 87.66±6.44 and Hip circumference mean values are 94.43±6.44 (ratio 0.928±0.04) which is higher along with mean serum ferritin values in comparison with healthy participants. In this study, relative to the normal weight men and women of age 30-65 years (BMI≤25), the overweight men and women (BMI: 25-29.9) and the obese men and women (BMI≥30) are 3-5 times risk with serum elevated ferritin levels. In detail explanation, a high body mass index implies a high energy intake. As high calorie intake or high nutritional iron intake is proportional to energy intake. Respondents with high BMI than normal have high calorie intake and iron intake, which eventually regulates body iron stores and serum ferritin [21]. Additionally, research conducted in Chinese middle aged and older aged men and women demonstrates elevated serum ferritin predicts abdominal trunk fat mass [22]. In one of the study conducted plasma ferritin as similar to C reactive protein acts as inflammatory marker which shows positive association with BMI and abdominal obesity. In our study percentage of elevated ferritin in healthy control subjects founded in men is 5.4 and women is 7.56 and among these elevated ferritin percentage respondents, mean BMI was 23.8 kg/m2 as shown in table 2 and figure 2 which shows positive association with BMI and components of metabolic syndrome but not with fasting blood glucose levels and HOMA-IR. This phenomenon may be due to the diet iron plays significant role in regulation of body iron stores and serum ferritin which supported by one of the study, in which mice fed with iron rich diet resulted in altered body mass and noted decreased synthesis of adiponectin by adipocyte [23]. The number of respondents in impaired fasting glucose levels with

elevated ferritin is 24% in men and 27% (mean BMI 24.8 Kg/m2) (shown in table 2 and figure 3) in women which is higher in contrast with healthy participants which demonstrates elevated ferritin levels may impairs glucose utilization by damaging insulin function with Higher BMI. In this study the mean serum ferritin (180.80± 19.4ng/ml) and mean values of waist hip ratio 0.928±0.04 in impaired fasting glucose respondents positively associated with fasting plasma insulin levels, various components of metabolic syndrome and HOMA-IR as shown in table 1. In this study the percentage of respondents who accepts at least equal or greater than 3 components of metabolic syndrome in impaired fasting glucose group was founded significantly greater than healthy participants (men=22.65 %(29), women=21.97%(20) as shown in table 3. These findings are supported by research conducted in obese women with abdominal (android fat) distribution is more insulin resistance than those with peripheral (gynecoid) obesity in contrast with non-obese women [10, 24]. In this study mean serum ferritin levels in impaired fasting glucose respondents leads to risk of developing type 2 diabetes mellitus. In normal participants elevated percentage of ferritin levels indicates hyperferritinnemia occurs before elevation of plasma glucose concentration as it predicts burden on insulin receptors. The possible mechanism, free iron is catalyst in the formation of hydroxyl free radicals, which is powerful pro oxidant that damages cellular bi-lipid layer membranes, oxidation of glycoproteins and altering nucleotide sequencing in genes [25]. Therefore the hypothesis that is established is free iron contributing to the formation of hydroxyl radicals leads to insulin resistance eventually to type 2 diabetes mellitus with chronic complications [26]. In this mean serum ferritin correlates with mean fasting insulin levels (7.55 ±1.11), mean values of calculated insulin resistance marker HOMA-IR (2.21 (2.9-3.95) and mitigates mean HDL-Cholesterol values (45.2 ± 5.8) in comparison with healthy control participants as depicted in table 1, these findings supports previous studies[27,28]. The decreasing level of HDL Cholesterol indicates chance for development of cardiovascular risk.

The highest category of serum ferritin levels was independently associated higher presence of metabolic syndrome in contrast with lowest category similar correlation founded in studies conducted in china and Taiwan [10, 29, 30]. This association was very significant when adjusted with an inflammatory biomarker such as C-reactive protein. Previous findings and recent cross sectional prospective studies demonstrates elevated serum ferritin significantly relates to various components of metabolic syndrome [19, 31-34] and eventually lead to cardiovascular risk [35]. These study findings recommend elevated serum ferritin levels predict as early marker for development of insulin resistance, metabolic syndrome and persistently leads to cardiovascular diseases. As in this study mean HDL-Choelsterol levels are low in impaired fasting glucose respondents with high mean serum ferritin levels which is distinguishing difference with healthy particiapnts. Even after many researchers conducted upon elevated ferritin in relationship with insulin resistance though there is no clear explanation for elevated ferritin is the main consequence to metabolic syndrome but recently research explains the hypothesis, called the iron dysregulation and Dormnant microbes (IDDM) has been proposed to support a host of metabolic diseases. In body, iron metabolism reveals conservation of iron and recycling is controlled by hepcidin[36]. But, hepcidin homeo statically regulated by iron availability and erythropoetic activity [37].

Table 1 shows mean of serum ferritin relates components of metabolic syndrome and components of insulin resistance syndrome (HOMA-IR) among the participants aged ≥30 years in healthy and impaired fasting glucose subjects

Characteristics	Healthy subjects* Group-1	Impaired fasting glucose * (Group-2)	*P*(analyis)
Age (years)	46.0±4.8	50.05±5.5	<0.001
Sex (M/F)	259/119	128/91	
Systolic blood pressure (mmHg)	117.5±9.4	132.8+11.2	<0.001
Serum insulin uU/ml	5.8± 0.88	7.55 ±1.11	<0.001
Fasting blood glucose (mg/dl)	90±9.8	119±12.0	<0.001
Total cholesterol (mg/dl)	140.50 ± 15.12	182.2±22.56	<0.001
HDL cholesterol (mg/dl)	51.43± 4.4	45.2 ± 5.8	<0.001
Triglycerides (mg/dl)	110±10	143.5±12.4	<0.001
BMI (kg/m2)	20.16±2.13	23.96 ±2.63	<0.001
Waist circumference (cm)	83.44+4.55	87.66±6.44	<0.001
Waist hip ratio	0.928±0.04	0.89±0.03	<0.001
serum ferritin (ng/ml)	108.0	180.80	<0.001
HOMA -IR	1.28 (0.81-1.75)	2.21 (2.9-3.95)	<0.001
HbA1C	5.2±0.2	5.9±0.4	<0.001
Transferrin saturation %	28.9(0.3)	26.7(0.5) †	<0.001
TGL/HDL-C ratio	2.156±0.4	3.17±0.6	<0.001

Table 2 shows mean serum ferritin concentrations in adults and percentage of individuals with elevated ferritin concentrations

Characteristic	Sample size (n)	mean ferritin (ng/ml)	Percentage with elevated ferritin concentration
Total	607	144.4±18.00	14.82±2.4 (90)
Men	387	167.15± 19.05	11.62±3.5 (45)
Women	220	121.65± 16.95	15.90±1.3 (35)
Healthy participants Normal			
Men	259	118 ±16.2	5.4 ±1.1 (14)
Women	119	98 ±14.2	7.56±0.9 (9)
Impaired fasting glucose subjects			
Men	128	216.3 ±21.9	24.21±2.4 (31)
women	91	145.3 ±17.7	27.57±1.7 (26)

Table 3 shows percentage of elevated ferritin concentration with those respondents accepts criteria > components of metabolic syndrome

Characteristic	Sample size (n)	Percentage of elevated ferritin in respondents those who at least accepts ≥3 components of metabolic syndrome	Total Percentage with elevated ferritin concentration
Total	607	11.03(67)	14.82±2.4 (90)
Men	387	10.59(41)	11.62±3.5 (45)
Women	220	11.81(26)	15.90±1.3 (35)
Healthy participants Normal			
Men	259	4.63 (12)	5.4 ±1.1 (14)
Women	119	5.04 (06)	7.56±0.9 (9)
Impaired fasting glucose subjects			
Men	128	22.65 (29)	24.21±2.4 (31)
women	91	21.97 (20)	27.57±1.7 (26)

Table 4 shows linear regressions of fasting concentrations of plasma insulin, glycosylated hemoglobin, and fasting blood glucose on log-transformed serum ferritin concentrations among participants aged in adult participants

Independent variables	Rank correlation	Unadjusted in (ferritin)	R^2	P
Men				
Fasting insulin uIU/ml	0.13	0.0025±0.0004	0.022	<0.001
Glucose mg/dl	0.11	0.0796±0.0113	0.020	<0.001
Glycosylated Hb%	0.01	0.0790±0.0202	0.007	<0.001
women				
Fasting insulin uIU/ml	0.19	0.0039±0.0005	0.026	<0.001
Glucose mg/dl	0.30	0.1524±0.0164	0.046	<0.001
Glycosylated Hb%	0.25	0.2373±0.0164	0.041	<0.001

Table 5 shows linear regressions of fasting concentrations of plasma insulin, glycosylated hemoglobin, and fasting blood glucose on log-transformed serum ferritin concentrations among participants aged in adult participants

Independent variables	Rank correlation	adjusted in (ferritin)	R^2	P
Men				
Fasting insulin uIU/ml	0.13	0.0016±0.0005	0.022	<0.001
Glucose mg/dl	0.11	0.0683±0.0137	0.020	<0.001
Glycosylated Hb%	0.01	0.0483±0.0234	0.007	<0.001
women				
Fasting insulin uIU/ml	0.19	0.0028±0.0005	0.026	<0.001
Glucose mg/dl	0.30	0.0767±0.0155	0.046	<0.001
Glycosylated Hb%	0.25	0.0767±0.0298	0.041	<0.001

Table 6. odd ratio in impaired fasting glucose and healthy participants (respondents without all 3 components)
Percentage of elevated ferritin levels comparsion in both groups

Men		Women
Odd ratio	5.59	4.88
95 CI	2.8519-10.968	2.158-11.0747
Z-Statistic	5.010	3.804
P	<0.0001	0.0001

Table 7. odd ratio in impaired fasting glucose group and healthy participants (in respondent accepting ≥ components of metabolic syndrome)
Percentage of elevated ferritin levels comparsion in both groups

Men		Women
Odd ratio	6.029	5.3052
95 CI	2.958-12.288	2.0325-13.8472
Z-Statistic	4.946	3.409
P	<0.0001	0.0007

Figure 2: Shows percentage of individuals with elevated serum ferritin levels in healthy subjects. According world health organization, iron overload defined as elevated ferritin levels >200 ng/dl for men and 150 ng/dl in women

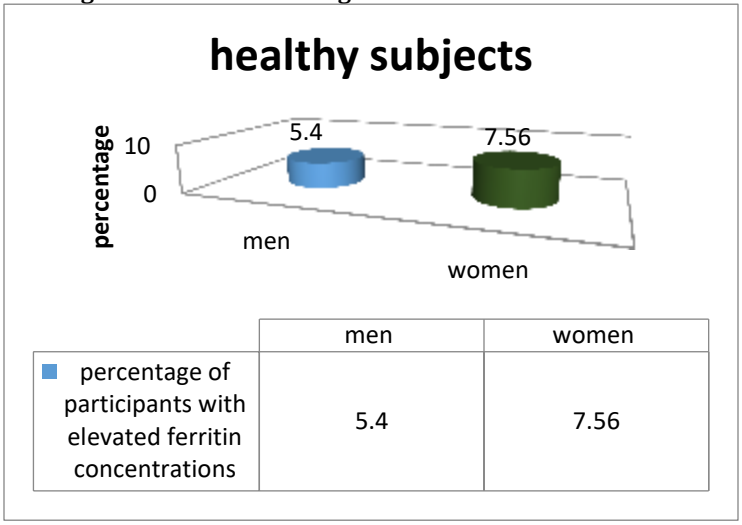
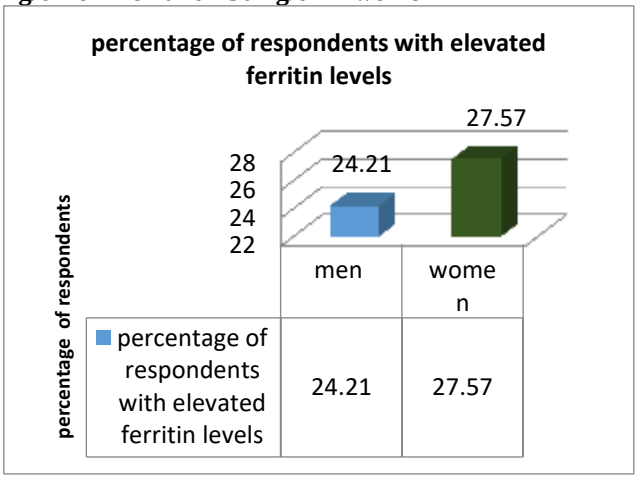


Figure 3: Shows percentage of respondents with elevated serum ferritin levels in impaired fasting glucose subjects. According world health organization, iron overload defined as elevated ferritin levels >200 ng/dl for men and 150 ng/dl in women



Consequently, iron in a healthy person is in the state of metabolic balance. There are several factors that grounds for release of free iron from the serum ferritin may results in iron dysregulation, such as a nutritional stress and oxidative stress [38].

In conditions of high iron, single hepcidin is not adequate to regulate iron homeostasis [39]. Elevated serum due to iron dysregulation results in cell death and microbial reactivation, further to inflammation to release inflammagens such as lipopolysaccharide and lipo-teichoic

acids which damages pancreatic beta cells result to insulin resistance syndrome and metabolic disorders [36].

In this study findings confirm that elevated serum ferritin may result increase in various components of metabolic syndrome and elevated HOMA-IR which is supported by recent study [40] and systematic review and metanalysis shows elevated ferritin relates to risk of metabolic syndrome[41]. The odd ratio in men 5.59 (95% CI: 2.8519-10.9680) and women is 4.88 (95 CI%: 2.1582-11.0747) as depicted in table 6 which shows more significantly exposure of serum ferritin positively leads to disease with impaired fasting glucose subjects in contrast with healthy participants. The odd ratio shows very significant positive correlation with risk of metabolic syndrome in comparison of two groups as shown table 7 (men=6.02: women 5.30) (r=+1 table 4&5). Karl's pearson coreelation coefficient r (+) 1 indicates perfect positive correlation which shows ferritin is independent variable as shown in table 4 and 5.

CONCLUSION

In conclusion, we found significant correlation between elevated serum ferritin and components of metabolic syndrome in impaired fasting glucose respondents compared with healthy participants. The percentage of respondents with elevated serum ferritin is more in impaired fasting glucose individuals in comparison with healthy subjects. In healthy participants of elevated ferritin levels, mean BMI is higher than participants with normal ferritin levels. Elevated serum ferritin level correlates with components of metabolic syndrome in impaired fasting glucose respondents. Additionally, before impairment of glucose utilization, serum ferritin may be elevated and predicts burden on insulin function. Serum ferritin is correlated with insulin resistance marker in men and women by calculating HOMA-IR. Further, prospective research studies should be conducted to find out pathological phenomenon and to confirm whether serum ferritin predicts metabolic disorders relates to impairment glucose utilization.

DISCLOSURE

The funding for this research is self.

CONFLICT OF INTEREST

The authors declare that they have no competing interests

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