Association between Serum Ferritin Levels and Risk of the Metabolic Syndrome in Urban Area of District Vadodara, Gujarat.

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Abstract: Ferritin is one of the key proteins regulating iron homoeostasis and is a widely available clinical biomarker of iron status. Elevated Serum Ferritin (SF) levels have been reported to be associated with several metabolic Disorders (MetS). Individual with highest SF were more likely to consume high amount of animal protein, betel nuts and lower amount of carbohydrates, compared with individual with lowest amount of SF. Association among serum Ferritin and metabolic syndrome compared by metabolic syndrome score(MSS). Results will highlight the crucial role of serum Ferritin level in health of people in Vadodara.

Keywords **- Serum Ferritin (SF), metabolic Disorders (MetS), metabolic syndrome score (MSS) iron homeostasis, biomarker.**

I. INTRODUCTION

Normal physiological process requires iron as vital important metal [1], and many metabolic processes such as oxygen transport, DNA synthesis, and electron transport done by this ubiquitous metal.[2]. Iron deficiency is one of the most prevalent nutrient disorders. It affects approximately two billion people worldwide and is the most common cause of anemia (WHO) [3]. Ferrous iron combines with apoferritin and is stored by ferritin in many organisms in a form of serum ferritin [4]. As an excessive accumulation of ferritin can increase the concentration of catalytically active free iron, and serum Ferritin concentration has been considered a possible risk factor of various chronic diseases including cancer, cardiovascular disease, diabetes, and essential hypertension, in which oxidative stress is closely involved in pathogenesis [5-9]. Newly research conducted on serum Ferritin concentration and MetS in all over the world. Though, the findings for the association of MetS and serum Ferritin concentration are still being debated because of variation across the studies. [14-17] .The present study has been done to investigate the association between MetS and its components and serum Ferritin concentration in adult barodian men and women aged 20 and above.

II. MATERIALS AND METHOD

1. Study details

Research subjects of a retrospective study were classified by gender and by age into 20~29 years, 30~39 years, 40~49 years, 50~59 years, 60~ 69 years, and 70 years and above. Data was collected for 55 days. We limited the analyses to adults aged ≥20 years (n = 59). We excluded two subjects of metabolic syndrome measurement whose data were missing but it is not affect on final results. Ferritin level of subject Estimated and Clinical biochemistry data collected from the Parul Sevashram Hospital, Limda, Waghodia, and Vadodara.

2. Laboratory measurements

The data were obtained from 8 hours fasting blood samples. Heparinized whole blood was collected for on-site measurement of hemoglobin. Peripheral venous blood samples were collected in tubes containing EDTA centrifuged at 40 and stored serum at -800 until analysis. Hemoglobin is estimated by semi auto cell-counter of Bensphera-H51, 5-part Hematology analyzer. Clinical biochemistry tests done by fully auto analyzer of Erba EM 360.

Table1: Method used for determination of different tests.

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Name of test	Method used for determination
Total cholesterol	Enzymatic colorimetric method [26].
Serum cholesterol	End point estimation using cholesterol oxidase and peroxidise[26]
Serum triglyceride	Enzymatic colorimetric method [27].
LDL cholesterol	Direct enzymatic method[28]
HDL cholesterol	Direct enzymatic method[29]

Serum iron	Electrochemiluminescence immunoassay [25]
Serum Ferritin	Electrochemiluminescence immunoassay [25]
TIBC	Electrochemiluminescence immunoassay [25]
Hemoglobin	Shale's method [30]

3. Serum Ferritin measurement

Serum Ferritin was measured with a AU2700/5400 Beckman Coulter Chemistry Analyzers. (Manufactured by: Beckman Coulter, Inc., 250 S. Kraemer Blvd. Brea, CA 92821, USA) [24]. Antibody-coated latex beads aggregating is latex agglutination reactions which occur as a result sufficient quantity of antigen is present in [25]. This is measured spectrophotometrically on Beckman Coulter Chemistry Analyzers.

4. Definitions of iron deficiency anemia (IDA) and iron overload

Iron status was evaluated by serum iron, transferring saturation and serum Ferritin concentrations [14]. Serum Ferritin was measured using a commercially available Electrochemiluminescence immunoassay and was quantitated by the Beckman coulter [25]. Hemoglobin was measured by semi-auto cell counter Benesphera-H51, 5-part Hematology analyzer. Serum iron and TIBC were measured by fully auto analyzer ErbaEM 360. Percentage transferrin saturation (TS) was calculated by serum iron/TIBC *100%. Iron deficiency was considered if ≥2 abnormal values of 3 indicators of iron status: SF <12 ng/mL, TS <15% and hemoglobin <13 mg/dL in men and <12 mg/dL in women. Hemoglobin cut-off point (men <13 mg/dL and women <12 mg/dL) was used to define anemia. Iron overload was defined as serum Ferritin >300 ng/mL for men and >200 ng/mL for women [15].

5. MetS and MSS

MetS was defined using the diagnostic criteria of the National Cholesterol Education Program (NCEP) based on common clinical measures including TG, HDL-C, and blood pressure. TG over 150 mg/dL was set as the criterion for elevated TG. The criteria for reduced HDL-C were HDL-C of less than 40 mg/dL and 50 mg/dL for men and women, respectively. Systolic blood pressure over 130 mmHg or DBP over 85 mmHg or medication were set as the criteria for elevated blood pressure. The presence of defined abnormalities in any three (FBG, fasting blood sugar and WM, waist measurement are excluded.) measures indicated a diagnosis of MetS. The presence of abdominal obesity indicates to metabolic syndrome score (MSS), reduced HDL-C, or elevated blood pressure, elevated FBG, elevated TG. Subjects without any of these five risk factors received an MSS 0, and those with one, two, three of the risk factors received an MSS score of 1, 2, 3 respectively as per Yoon H et al 2015.[16].

6. Statistical Data analysis

The results obtained were statistically analyzed by using continuous variables were presented as mean with standard deviations and then compared between different groups of the study by applying Independent 't' test. The results were expressed as Mean ± SD and were taken as significant when the probability (p,0.05), (p,0.001) as the percentage of the observing values of 't' at a particular degree of freedom and Pearson's correlation analysis was performed [31].

III. RESULTS

1. General characteristics of research subjects

General characteristics of the research subjects are shown in Table 2. According to the classification of risk factors for coronary artery disease and the MSS guidelines 59 subjects were classified as MSS 0, MSS 1, MSS 2, MSS 3 respectively. The prevalence rate of MetS was 1(1.69%) of the 59 patients (men, 0%; women, 40.3.443%). The mean value of serum Ferritin levels was 229.12±327.70 ng/mL (men, 282.2±282.80; women, 174.08±360.20ng/mL).

Table 2. Common attributes

Variables	Category	total(n= 59)	men(n=30)	women(n=29)
age(years)	20~29	12	5	7
	30~39	11	3	8
	40~49	11	5	6
	50~59	11	7	4
	60~69	12	8	4
	>70	2	2	0
MSS	0	40	20	20
	1	11	7	4
	2	7	3	4
	3	1	0	1
Mets	MSS<3	58	30	28
	MSS>=2	8	3	5
	MSS=3	1	0	1
SBP(mm/hg)		140.47±1.12	138.54±24.33	126±21.10
DBP(mm/hg)		84.24±2.68	83.09±15.36	70.28±18.52
TC(mg/dL		194.5±9.91	191.5±18.89	197.5±38.72
TG(mg/dL)		173.37±33.37	122±49.21	213.14±115.94
HDL-C(mg/dL)		41.63±0.020	43.2±9.16	38.7±9.20
Ferritin(ng/ml)		229.12±327.70	282.2±282.80	174.08±360.20

FOOT NOTE: M±SD shows mean and standard deviation value of blood pressure, total cholesterol, total triglyceride and Ferritin value of patients.

Abbreviation: MSS, metabolic syndrome score; MetS, metabolic syndrome; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; TG, triglyceride; HDL-C, high density lipoprotein cholesterol .

2. Serum Ferritin levels by subject characteristics

Serum Ferritin levels by subject characteristics were shown in Table 3. In the men group, serum Ferritin levels were associated with age (p =0.167), TC (p ＜0.001), in the women group, serum Ferritin levels were associated with age (p ＜0.001), TC (p =0.22).

Table 3. Serum Ferritin levels according to general characteristics

		men (n=30)		women (n=29)	
Variables	Category	Ferritin(ng/ml)	p-value	Ferritin(ng/ml)	p-value
age(years)	20~29	93.8±129.75	0.167	6.17±4.81	<0.001
	30~39	476.46±386.45		26.52±42.19	
	40~49	300.36±354.62		661.61±518.57	
	50~59	388.14±270.52		53.33±43.39	
	60~69	241.75±137.469		105.33±67.85	
	>70	19.05±8.05		0.01±0.01	
TC(mg/dL)	<200	307.7±9.43	<0.001	173±11.11	0.22
	>=200	518.9±0.10		246.5±118.05	

FOOT NOTE: As shown above age group 20~29 in both man and women have low serum Ferritin level and it is cleared that increasing total cholesterol is Directly Proportional to Ferritin.
Abbreviation: M±SD shows Mean and standard deviation value of total cholesterol (TC), and Ferritin value of patients.

3. Serum Ferritin levels by MetS and MSS

Means comparisons of serum Ferritin levels by MetS and MSS are shown in Table 4 and 5. In men and women groups, serum Ferritin levels were associated with all components of the MetS. After modification of associate variable of serum Ferritin levels, its mean value (M±SE) was significantly higher (p ＜0.001) in the MetS group (men, 00.0±0.0 ng/mL; women, 949.2±474.59 ng/mL) than the non- MetS group (men, 370.57±137.91 ng/mL; women, 274.20±284.84 ng/mL). In addition, serum Ferritin levels increased as MSS increased in men group (p=0.13), women (p < 0.001).

Table 4. Serum Ferritin levels according to MetS components

Variables	Category	total(n= 59)		men(n=30)		women(n=29)	
		Ferritin	P value	Ferritin	P value	Ferritin	P value
MSS	0	134.29±153.05	<0.001	230.56±284.71	0.13	38.021±21.39	<0.001
	1	206.935±108.44		304.21±152.54		109.66±64.35	
	2	606.55±418.58		565.2±281.76		674.9±555.41	
	3	474.6±237.295		0.0±0.0		949.2±474.59	
Mets	MSS<3	143.68±143.185	0.02	282.2±282.80	0.30	5.17±3.57	<0.01
	MSS=3	474.6±237.295		0.0±0.00		949.2±474.59	
BP(mm/hg)	Normal BP	122.53±110.24	<0.001	239.9±224.6	0.56	5.17±3.57	0.02
	Elevated BP	380.01±73.59		345.92±274.92		414.11±422.16	
TG(mg/dL)	normal TG	171.67±167	<0.001	338.75±61.45	0.36	4.5±2.0	0.1
	elevated TG	615.48±86.28		565.2±281.76		737.76±499.50	
HDL-C(mg/dL)	Normal HDL-C	269.4±266.8	0.007	536.2±283.71	0.40	2.6±1.29	0.12
	reduced HDL-C	598.17±215.5		382.25±136.65		814.1±5111.11	

Foot note: serum ferritin levels according to MetS higher in a group of men and women it is low.
Abbreviation: MSS, metabolic syndrome score; MetS, metabolic syndrome; TG, triglyceride; HDL-C, high density lipoprotein cholesterol. Elevated triglyceride is defined as TG ≥150 mg/dL, Normal is defined as HDL-C≥40 mg/dL in men or ≥50 mg/dL in women, Reduced HDL-C is defined as HDL-C ＜40 mg/dL in men or ＜50 mg/dL in women, Normal is defined as SBP＜130 mmHg or DBP＜85 mmHg, Elevated blood pressure is defined as SBP≥130 mmHg or DBP≥85 mmHg.

Table 5. Comparisons of serum Ferritin levels for MetS and MSS

Variables		men (n=30)		women (n= 29)	
		Ferritin(ng/ml)	p-value	Ferritin(ng/ml)	p-value
MSS	0	230.56±284.71	0.13	38.021±21.39	<0.001
	1	304.21±152.54		109.66±64.35	
	2	565.2±281.76		674.9±555.41	
	3	0.0±0.0		949.2±474.59	
non-MetS		370.57±13791	<0.01	274.20±284.84	0.05
MetS		00.0±0.0		949.2±474.59	

Foot note: 'Table 5' Shows relation between non-MetS verses MSS shows P< 0.01

IV. DISCUSSION

Serum Ferritin is used as a clinical biomarker to evaluate iron status, and elevated serum Ferritin concentration has been reported to be associated with the risk factors of cardiovascular disease such as diabetes mellitus, insulin resistance, and hypertension [6-9]. Each component of MetS is a risk factor for coronary artery disease, and MetS in which the components occur in a cluster with resistance to insulin is a useful indicator to identify groups at a high risk for cardiovascular disease [17,18]. Among previous studies on serum Ferritin concentration and MetS, Li and colleagues reported that serum Ferritin levels were positively associated with MetS in both Chinese men and women [12]. Moreover, Vari and colleagues reported that serum Ferritin levels were positively associated with MetS in French men and premenopausal women and postmenopausal women [10]. Other side report of Jehn and colleagues in a study with US adults shows positively correlation of serum Ferritin levels and MetS women group, but not in men group [19]. Moreover, Jeong and colleagues reported in a study on Korean adults that serum Ferritin levels were positively associated with MetS in men, but not in women [20]. In addition, Ryu and colleagues reported that serum Ferritin levels were not associated with MetS in both men and women [21]. The association between serum Ferritin concentration and individual components of MetS varies between countries and races. In the present study results, although individual metabolic syndrome components may not be associated with serum Ferritin concentration according to sex, increases of its components were positively associated with the serum Ferritin concentration. In conclusion, MetS was associated with an increase in serum Ferritin levels in barodian adults among both of the gender-based groups (men and women).

V. LIMITATIONS OF THE STUDY

This study has several limitations. First, serum Ferritin is an acute-phase reactant and may be increased under inflammatory conditions. We could not adjust for CRP as an inflammatory marker because it was not checked, the information for dietary intake which may influence body iron stores, is not concluded. Second thing, we could not analyze comprehensively the association of serum Ferritin and MetS, due to lack of information related to fasting blood sugar and waist measurement as a marker of body iron status because it is independent factor in this association[23]. Third, because this study was a cross-sectional study, the ability to establish a causal relationship between serum Ferritin and the MetS and increase of its components was limited. Therefore, more accurate results might be obtained by performing a cohort study.

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REFRENCES

1. Heeney MM, Andrews NC. Iron homeostasis and inherited iron overload disorders: an overview. Hematol Oncol Clin North Am. 2004;18(6):1379-1403.
2. Crichton RR. Inorganic biochemistry of iron metabolism. 3rd ed. New York: Ellis Horwood; 1991. p.29-58.
3. (2000) World Health Organisation (WHO) The global picture of anaemia. Technical report series 2000: Report of a WHO scientific group, Geneva: 1523.
4. Cook JD, Flowers CH, Skikne BS. The quantitative assessment of body iron. Blood. 2003; 101(9):3359-3364.
5. Halliwell B. Oxidative stress, nutrition and health. Experimental strategies for optimization of nutritional antioxidant intake in humans. Free Radic Res. 1996;25(1):57-74.
6. Jiang R, Manson JE, Meigs JB, Ma J, Rifai N, Hu FB. Body iron stores in relation to risk of type 2 diabetes in apparently healthy women. JAMA. 2004; 291(6):711-717.
7. Jehn ML, Guallar E, Clark JM, Couper D, Duncan BB, Ballantyne CM, et al. A prospective study of plasma ferritin level and incident diabetes: The atherosclerosis risk in communities (ARIC) study. Am J Epidemiol. 2007; 165(9):1047-1054.
8. Piperrno A, Trombini P, Gelosa M, Mauri V, Pecci V, Verqani A, et al. Increased serum ferritin is common in men with essential hypertension. J Hypertens. 2002; 20(8):1513-1518.
9. Cho YS, Kang JH, Kim SA, Shim KW, Lee HS. Association of serum ferritin and abdominal obesity and insulin resistance. J Korean Soc Study Obes. 2005;14(2):76-81.
10. Vari IS, Balkau B, Kettaneh A, Andre P, Tichet J, Fumeron F, et al. Ferritin and transferring are associated with metabolic syndrome abnormalities and their change over time in a general population: Data from an Epidemiological Study on the Insulin Resistance Syndrome (DESIR). Diabetes Care. 2007; 30(7):1795- 1801.
11. Ferrannini E. Insulin resistance, iron and liver. Lancet. 2000; 355(9222):2181-2182.
12. Li J, Wang R, Luo D, Li S, Xiao C. Association between serum ferritin levels and risk of the metabolic syndrome in Chinese adults: a population study. PLoS One. 2013;8(9):e74168. doi: 10.1371/journal.pone.0074168.
13. Raghavendra BM, Bharath MS, Veeranna Gowda KM, Ahmed MF, Yashavanth HS. Study of serum ferritin as a component of metabolic syndrome. IJSR. 2014;3(12):2383-2385.
14. Sun L, Franco OH, Hu FB, Cai L,Yu Z, Li H et al. Ferritin concentrations, metabolic syndrome, and type 2 diabetes in middle-aged and elderly chinese. J Clin Endocrinol Metab. 2008; 93:4690-6. doi: 10.1210/jc.2008-1159

15. Looker AC, Dallman PR, Carroll MD, Gunter EW, Johnson CL. Prevalence of iron deficiency in the United States. JAMA. 1997; 277:973-6. doi: 10.1001/jama.1997.03540360 041028.
16. Yoon H, Kim GS, Kim SG, Moon AE. The Relationship between metabolic syndrome and increase of metabolic syndrome score and serum vitamin D levels in Korean adults: 2012 Korean National Health and Nutrition Examination Survey. J Clin Biochem Nutr. 2015;57(1):82-87.
17. Meigs JB. Invited Commentary: Insulin resistance syndrome syndrome X multiple metabolic syndrome a syndrome at all factor analysis reveals patterns in the fabric of correlated metabolic risk factors. Am J Epidemiol. 2000;152(10):908-911.
18. Grundy SM. Metabolic syndrome: a multiplex cardiovascular risk factor. J Clin Endocrinol Metab. 2007;92(2):399-404.
19. Jehn M, Clark JM, Guallar E. Serum ferritin and risk of the metabolic syndrome in US adults. Diabetes Care. 2004;27(10): 2422-2428.
20. Jeong DW, Lee HW, Cho YH, Yi DW, Lee SY, Son SM, et al. Comparison of serum ferritin and vitamin d in association with the severity of nonalcoholic fatty liver disease in Korean adults. Endocrinol Metab. 2014;29(4):479-488.
21. Ryu SY, Kim KS, Park J, Kang MG, Han MA. Serum ferritin and risk of the metabolic syndrome in Korean rural residents. J Prev Med Public Health. 2008;41(2):115-120.
22 . Hyun Yoon, Jae Seong Go, Kang Uk Kim, Keon Woo Lee.The Association of Serum Ferritin and Metabolic Syndrome and Metaboli Syndrome Score in Korean Adults, Korean J Clin Lab Sci. Vol. 48, No. 4, December 2016,287-294,DOI: 10.15324/kjcls.2016.48.4.287.
23. Jung-Su Chang , Shiue-Ming Lin , Tzu-chieh Huang , Jane C-J Chao, Yi-Chun Chen, Wen-Harn , Chyi-Huey Bai , Serum ferritin and risk of the metabolic syndrome: a population-based study, Asia Pac J Clin Nutr 2013;22 (3):400-407, doi: 10.6133/apjcn.2013.22.3.07 .
24. Beckman Coulter, Inc., 250 S. Kraemer Blvd. Brea, CA 92821, USA . OSR Special BAOSR6x203.01 OSR Special Chemistry 2010-03
file:///C:/Users/DELL/Desktop/ferritin/EN_FERRITIN%20bekmen%20coulter.pdf
25. Beckman Coulter, Inc., 250 S. Kraemer Blvd. Brea, CA 92821, USA .Bulletin 9075d
file:///C:/Users/DELL/Desktop/ferritin/ferritin%20bekmen%20coulter%20information.pdf
26. Coral cholesterol (CHOD- PAP enzymatic end point method) kit insert, Mfg. crest Biosystems, A division coral clinical systems.
27. Coral triglyceride (Glycerol phosphate oxidase colorimetric end-point method) kit insert, Mfg. crest biosystems, a division of coral clinical systems.
28. Accurate LDL cholesterol (direct enzymatic method) kit insert, Mfg. Lab care Diagnosis P. LTD.
29. Coral HDL cholesterol (Direct enzymatic method) kit insert, Mfg. coral Clinical systems.
30. Sharma S. Biochemical estimation of blood hemoglobin level by sahli's method, experiments and techniques in biochemistry 2007.
31. Gupta Sp. Statistical Methods, 39th revised edition sultan chand and sons 2010.