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SERUM FERRITIN LEVEL AS AN EARLY INDICATOR OF METABOLIC DYSREGULATION IN YOUNG OBESE ADULTS-A CROSS SECTIONAL STUDY

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ABSTARCT:

The aim of this study was to investigate the relationship between serum ferritin level and antioxidative status and metabolic dysregulation in young adult obese population. This cross-sectional study included three hundred subjects of either sex, grouped as obese and non-obese subjects. The Body Mass Index (BMI), serum ferritin, Iron, Total Iron Binding Capacity (TIBC), Fasting Blood Glucose (FBS), Total cholesterol (TC), High-Density Lipoprotein Cholesterol (HDL-C), Low-Density Lipoprotein Cholesterol (LDL-C), Triglyceride (TG), Glutathione (GSH), superoxide dismutase (SOD) activity and vitamin-C level was estimated. Analysis showed a significant alteration in all the parameters in obese adults. The correlation of ferritin level and BMI showed a positive correlation ($r=0.32$, $p<0.001$ respectively) with levels of FBS, SOD, TC, LDL and TG in obese individuals whereas, an insignificant correlation with Vit-C and GSH level was observed in obese individuals. The significant positive correlation of ferritin level with the metabolic parameters and some antioxidative parameters in obese individuals signifies the development of metabolic disorders. Therefore, estimation of serum ferritin level will be an important early indicator for the risk of developing metabolic disorders in young adults.

Key Words: Obesity, Serum Ferritin, Total Iron Binding Capacity, vitamin-C, glutathione, Super Oxide Dismutase, Metabolic dysregulation.

Introduction:

Obesity is a medical condition in which excess body fat accumulates to the extent that it may have an adverse effect on health, leading to reduced life expectancy and/or increased health problems. Obesity is a global health problem. Obesity increases the likelihood of various diseases, particularly heart disease, type 2 diabetes, obstructive sleep apnea, certain types of cancer and osteoarthritis (Xavier Pi-Sunyer 2009).

Ferritin, a ubiquitous intracellular protein that is essential for the regulation of iron homeostasis, is a widely available clinical biomarker used to estimate body iron status. Iron is a transition metal capable of causing oxidative stress and oxidative tissue damage by catalyzing the formation of free radicals (Xavier Pi-Sunyer 2009). However, increasing evidence has shown that moderately increased iron stores, described by an elevated serum ferritin level, are associated with various metabolic risk factors (Meghana 2015).

Ferritin is an acute-phase protein and its expression is up-regulated in conditions such as uncontrolled cellular proliferation, in any condition marked by excessive production of toxic oxygen radicals, and by infectious and inflammatory processes. Under such conditions ferritin up-regulation is predominantly stimulated by increased reactive oxygen radical production and by cytokines (Bresgen 2015; Koorts 2007). The major function of ferritin in these conditions is to reduce the bioavailability of iron in order to stem uncontrolled cellular proliferation and excessive production of reactive oxygen radicals.

Serum ferritin concentration is an indicator of the total amount of stored iron in the body (Beard et al. 2006) which is necessary to maintain the appropriate supply of oxygen and enzymes to cells and tissues (Yoon et al. 2011). Ferritin is also related to oxidative stress and excessive activation of reactive oxygen species, which contributes to the development of

insulin resistance, atherosclerosis and cardiovascular diseases (Galkina and Ley 2009; Galaris and Pantopoulos 2008). Overweight and obese people are at risk of developing certain serious complications like obstructive sleep apnea, ischemic heart disease, diabetes, and many others (Bozzini 2005). At the same time, it increases the likelihood of iron deficiency (ID) and iron deficiency anemia (IDA), leading to an escalating disease burden (Hutchinson 2016; Cepeda-Lopez et al. 2015; Baumgartner et al 2013; Zhao et al. 2015; Cheng et al. 2012), Iron deficiency (ID) and Iron deficiency Anemia (IDA) (Patel 2016; Khaskheli 2016).

The serum ferritin assay is the most commonly used blood test to diagnose ID or IDA in the developing countries. Several clinical studies have shown that elevated ferritin levels are associated with insulin resistance, diabetes mellitus (You and Wang 2005; Sun et al. 2008; Jee-Yon Lee et al. 2012). It was also reported that ferritin is a useful marker of metabolic syndrome including insulin resistance, reflecting the importance of oxidative stress as a cause of metabolic syndrome, especially in men (Iwanaga et al. 2014).

Iron is required for normal cell growth and proliferation. However, excess iron is potentially harmful, as it can catalyze the formation of toxic reactive oxygen species (ROS) via Fenton chemistry. Oxidation is a chemical reaction that can produce free radicals. In turn, these radicals can start chain reactions which can cause damage or death to the cell. Antioxidants terminate these chain reactions by removing free radical intermediates and inhibit another oxidation reaction (Beard et al. 2006). The antioxidants are often reducing agents such as thiols, ascorbic acid, or polyphenols. Ferritin is known to have biphasic roles in the body's response to oxidative stress; nevertheless, the role of ferritin in humans has not been fully clarified. As a pro-oxidant, iron plays a key role in converting poorly reactive free radicals into highly reactive free radicals. In contrast, the protective role of ferritin against oxidative

stress has been confirmed by several experiments. It was also reported that cells pretreated with ultraviolet radiation, which up-regulates the synthesis of ferritin, elevates anti-oxidant activity (Yoon et al.2011).

Vitamin-C (Ascorbic acid) is a redox catalyst which can reduce, and thereby neutralize, reactive oxygen species such as hydrogen peroxide. In cells, glutathione is maintained in the reduced form by the enzyme glutathione reductase and in turn reduces other metabolites and enzyme systems, as well as reacting directly with oxidants. Superoxide Dismutase is a class of closely related enzymes that catalyze the breakdown of the superoxide anion into oxygen and hydrogen peroxide, thereby, GSH and SOD acts as important antioxidant enzyme in humans (Lobo et al.2010).

Though it was well documented that, an elevated serum ferritin level is associated with various metabolic risk factors, the association of serum ferritin level with antioxidants and metabolic parameters in young adult population was not well documented. Therefore, this study will focus on correlating serum ferritin with iron, BMI, and other metabolic parameters to find out whether, serum ferritin as a true measure of early indicator metabolic imbalance in young adult obese population.

Materials and Methods:

This cross-sectional study was conducted after the approval from institutional ethical committee. The study group included obese subjects (n=150, BMI>30 kg/m²) among the staff and student community of the age group of 18-40 years of either sex with an informed and written consent duly signed by each participant. The control group included age and sex matched non-obese subjects (n=150, BMI<25 kg/m²) among the staff and student community

after an informed and written consent duly signed by each participant. The selection of participants was as shown in the flow chart (figure-1). The 450 subjects were listed in random order within age group of 18-40 years of either sex. From among 450 subjects considered for eligibility, 300 were finally eligible for participation after the exclusion of 150 subjects with informed consent. The sample size was adopted as per the literature (Hayder et al.2014). Adult subjects who are suffering from diabetes and hypertension were excluded.

All the required kits and reagents were procured from Sigma Aldrich (Merck), India. For the assessment of BMI, height and weight was measured with a standard height-weight scale, and BMI was calculated as weight divided by height squared. After the suitability for the study, under aseptic precautions, an overnight fasting sample of 6mL of venous blood was drawn in the morning. 2mL was put in an EDTA tube for hemoglobin (Hb) determination. 4ml of venous blood was collected without anticoagulant in appropriate sterile vials and serum was separated by centrifugation at 1500 rpm for 15minutes and stored at 4°C and used for further analysis.

The serum ferritin level was estimated using Enzyme Linked Immuno Sorbent Assay (ELISA) method as per the manufacturers guidelines (Khanna et al.2017). Glutathione level was estimated using 5,5'-dithiobis, 2-nitrobenzoic acid (DTNB) method (Tipple and Rogers 2012). Superoxide Dismutase activity level was estimated by Beauchamp and Fridovich method (Weydert and Cullen 2015). The vitamin C level was estimated using 2,4-Dinitrophenylhydrazine (DNPH) method (Rahman et al.2006). Serum iron was measured using enzymatic colorimetric method, and Hb was measured on automated analyzer Sysmex. The serum glucose level was measured by an enzymatic method (hexokinase) (Schulpis et al.2010). All these tests were done by the same technician in our central research laboratory.

Estimation of total cholesterol by CHOD-POD method: 3 test tubes blank (B), Test (T), and Standard(S) were taken. 5microlitre of the serum was added to T. 5microlitre of standard was added to S. 100microlitre of reagent was added to each test tube, mixed and incubated for 5mins at 37°C. The absorbance was taken at 505 nm against blank spectroscopically and calculated as, Cholesterol= (absorbance of test/absorbance of standard) x conc. of standard.

Estimation of HDL cholesterol by precipitation end point method: 3 test tubes Blank (B), Test (T) and Standard (S) were taken. 25microlitre of serum was added to T. 25microlitre of standard was added to S. 500microlitre of reagent was added to each test tube, mixed and incubated for 10mins at 37°C. The absorbance was taken at 505nm against green filter and calculated as HDL-Cholesterol= (absorbance of test/absorbance of standard) x conc. of standard x 2

Estimation of Triglyceride by GPO-POD method: 3 test tubes Blank (B), Test (T) and Standard(S) were taken. 10microlitre of the serum was added to T. 10microlitre of standard was added to S. 1000microlitre of reagent was added to each test tube, mixed and incubated for 10mins at 37°C. The absorbance was taken at 505nm against reagent blank and calculated as, Triglycerides=absorbance of test x absorbance of standard x conc. of standard.

LDL-C was calculated indirectly by the method of Fried Wald as $LDL = TG - (HDL + VLDL)$

Data Analysis:

The data was analyzed using student's t-test for statistical significance between different parameters in obese and non-obese individuals and the correlation of ferritin with other antioxidative parameters by Pearson correlations using SPSS software (Version-16 SPSS Inc., Chicago, IL). P value less than 0.05 was considered the level of significance.

Results:

The baseline characteristics of all the eligible and recruited subjects were given in table-1.

Demographic Characteristics of the Participants: Overall, 54.0% of the subjects were males and 46.0% were females (table-1). 46.0% of the subjects were 18–24 years old, 36.0% were 25-35 years and 17.0% were belongs to 36-40 years old. The majority of the subjects were unmarried (67.0%). 60.0% of the participants were studying and 37% were employed. Overall, 82% of the participants were at the undergraduate level and only 8% were studied up to plus two level(table-1).

Clinical Characteristics of the Participants: The study did not include the patients with hypertension, diabetes or congenital heart disease(table-1). But, 3.0% of the participants were former smokers, 12.0% were recurrent smokers, 42.0% were very rarely smokers and 49.0% of the participants were never smokers.

Comparison of test variables between obese and nonobese showed a significant difference except fasting blood glucose level. The Hb concentration, Iron concentration, Total Iron Binding Capacity was found to be significantly decreased ($p=0.0001$) in obese subjects. Whereas, Serum ferritin level, Vitamin-c, Glutathione and SOD were significantly increased ($p=0.0001$) in obese subjects as compared to nonobese subjects (table-2).

The correlation of body mass index with metabolic and antioxidative parameters was represented in table-3. The correlation of BMI of obese individuals showed significant difference with respect to Hb ($r=-0.72$, $p<0.001$), Iron concentration ($r=-0.81$, $p<0.001$), total iron binding capacity ($r=-0.82$, $p<0.001$), Ferritin ($r=0.79$, $p<0.001$), Glucose ($r=0.68$, $p<0.001$), SOD ($r=0.35$, $p<0.001$) respectively. Whereas, the concentration of Vitamin-C ($r=0.18$, $p>0.05$) and Glutathione ($r=0.17$, $p>0.05$) did not show any significant correlation with BMI of obese individuals.

The total cholesterol, LDL-C, and TG concentrations were positively correlated with serum ferritin levels. HDL-C values were negatively associated with serum ferritin levels.

The results of correlation of ferritin with metabolic and antioxidative parameters was shown in table-4. The correlation of ferritin level of obese individuals showed significant difference with respect to BMI ($r=-0.81$, $p<0.001$), Iron concentration ($r=-0.82$, $p<0.001$), total iron binding capacity ($r=-0.79$, $p<0.001$), Hb concentration ($r=-0.58$, $p<0.001$) and SOD ($r=0.39$, $p=0.001$), total cholesterol ($r=1.23$, $p<0.001$), HDL ($r=-1.62$, $p<0.001$), LDL ($r=1.95$, $p<0.001$), Triglyceride ($r=1.23$, $p<0.001$) respectively. The concentration of Vitamin-C ($r=0.16$, $p>0.05$) and Glutathione ($r=0.18$, $p>0.05$) did not show any significant correlation with ferritin level in obese individuals.

Discussion:

In the present study, we found that metabolic and antioxidative parameters gets altered in obese subjects. The Hb concentration, Iron concentration, Total Iron Binding Capacity was significantly decreased in obese subjects. Whereas, Serum ferritin level, Vitamin-c, Glutathione and SOD were significantly increased. But, fasting blood glucose level did not exhibit significant variation.

The correlation between ferritin level and metabolic and antioxidative parameters showed a significant difference. The correlation of ferritin level of obese individuals showed significant difference with respect to BMI, Iron concentration, total iron binding capacity, Hb concentration, SOD, total cholesterol, HDL, LDL and Triglyceride. Whereas, the concentration of Vitamin-C and Glutathione did not show significant correlation with ferritin level in obese individuals. These observations are in line with the previous reports (Nemi et

al.2013; Swaminathan et al.2007). However, this relationship was not established in the non-obese group.

Schulpis KH et al. 2010 reported that, obesity has been associated with iron deficiency in overweight children and adolescents, as well as in obese adults. It was also reported that cells enriched by ferritin showed an enhanced resistance to oxidative stress, whereas cells in which ferritin was down regulated had reduced resistance to oxidative stress (Schulpis et al. 2010). Ferritin is a universal intracellular protein that stores iron and releases it in a controlled fashion and acts as a buffer. Ferritin is found in most tissues as a cytosolic protein, but small amounts are secreted into the serum where it functions as an iron carrier. Plasma ferritin is also an indirect marker of the total amount of iron stored in the body, hence serum ferritin can also be used as a diagnostic test (Wang et al.2010).

It was reported that, ferritin concentrations increase drastically in the presence of an infection or cancer. Endotoxins are an up-regulator of the gene coding for ferritin, thus causing the concentration of ferritin to rise. By contrast, organisms such as *Pseudomonas*, although possessing endotoxin, cause plasma ferritin levels to drop significantly within the first 48 hours of infection. Thus, the iron stores of the infected body are denied to the infective agent, impeding its metabolism (Ong et al.2005).

Serum ferritin is a widely used marker of iron status in epidemiological studies and accurately reflects body iron stores in healthy individuals. Few studies have examined the relationship of body iron stores and indices of body fat distribution. Several lines of evidence support the link between body iron excess and insulin resistance. In the present study, we observed a significant increase in the level of plasma ferritin in obese individuals as compared nonobese subjects which is in accordance with other reports (Lecube et al.2008).

Antioxidant enzymes are the major defense system of cells in normal aerobic reactions. It was reported that, erythrocytes possess highly efficient antioxidant enzymes, such as SOD and glutathione peroxidase (GPx) compared to other cell types, as evidenced in our study, indicating the significant increase in the level of SOD and Glutathione in obese individuals which is in accordance with earlier such reports (Amit Kumar et al.2010).

Increased SOD activity in obesity may be linked to increased oxidative stress. Super oxide dismutase and Catalase are metalloproteins and accomplish their antioxidant function by enzymatically detoxifying the peroxides ($-OOH$), H_2O_2 and O_2^- respectively. It has been suggested to provide important pathway for H_2O_2 decomposition into H_2O and O_2 (Amit Kumar et al.2010). Similarly, a significant increase in the level of glutathione in obese individuals when compared with control was observed in the present study. Glutathione plays a pivotal role in protection of cells against oxidative stress. It can act as a non-enzymatic antioxidant by direct interactions of SH group with ROS or it can be involved in the enzymatic detoxification reactions for ROS as a coenzyme. The increased level of glutathione is to nullify the effect of oxidative stress in obesity (Rajpathak et al.2006).

Furthermore, the levels of vitamin C was also found to be increased significantly in obese subjects as compared to normal individuals. The increase in endogenous ascorbic acid may be to use as an antioxidant to protect the oxidative stress. Antioxidants in blood such as SOD significantly contribute to the total plasma antioxidant capacity (Rajpathak et al.2006) and are the first line of defense against oxidative stress (Paul et al.2014). Superoxide dismutase catalyzes conversion of superoxide anion to hydrogen peroxide (H_2O_2) (Tohru Fukai et al.2011) Hence, as expected, the results of the present study showed the increased levels of antioxidative parameters such as Vitamin C, Glutathione and SOD in obese as compared to

non-obese adults. Increase in these parameters might be a compensatory mechanism to scavenge excess free radicals as a result of oxidative stress in obesity. The possible mechanism underlying the anti-oxidative effect of ferritin is its capacity to bind to nitric oxide molecules, which has anti-oxidative properties, and to decrease lipid peroxidation by sequestering iron in solution.

Dyslipidemia, an excessive lipid in the blood, may begin in childhood, and is a causal factor of early atherosclerosis and premature cardiovascular diseases (CVD) in young adults (NCEP 1992; Daniels and Greer 2018; Kavey et al. 2003). Interestingly, in the present study, we identified an association of serum ferritin levels with lipid parameters such as TC, LDL-C, HDL-C, and TG were all significantly associated with serum ferritin levels.

Though it was well documented that ferritin's ability to act against oxidative stress is regulated by many proteins that sequester and mobilize iron (Nermi et al. 2013), the precise factors and pathophysiology that regulate the homeostasis between the anti-oxidant and pro-oxidant properties of ferritin was not clear. Hence, further studies are required by employing the participants from wider age group and large number of sample to explore the compensatory mechanism of ferritin in obesity leading to metabolic dysregulation.

In the present study, a significant increase in the level of metabolic and antioxidative parameters explains the increased oxidative stress in obese individuals. Also, the significant correlation of serum ferritin level with the parameters studied indicate the relation between oxidative stress and excessive activation of reactive oxygen species, which in turn contribute to the development of metabolic disorders. *With the limitation of the study, the lack of consideration of confounding factors, we conclude that, the* estimation of serum ferritin level

is clinically important as an early indicator for assessing the risk of developing metabolic disorders. Therefore, further studies need to be explored.

Conflict of interest statement: The authors declare that we have no conflict of interest.

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Table-1: Baseline characteristics of eligible study participants.

Characteristics	N	%
Demographic domain		
Gender		
Male	162	54
Female	138	46
Age (Years)		
18-24	142	47
25-35	106	36
36-40	52	17
Social domain		
Marital status		
Unmarried	190	63
Married	110	37
Employment status		
Studying	182	60
Employed	110	37
Unemployed	8	03
Educational status		
<High school	Nil	0
High school but <College	56	18
≥College	244	82
Clinical domain		
Hypertension	Nil	0
Congenital heart disease	Nil	0
Diabetes	Nil	0
Smokers		
Former smokers	08	03
Recurrent smokers	34	12
Very rarely smokers	128	42
Never smokers	130	43

Table-2: Comparison of test variables in obese and normal non-obese individuals. N =150 each.

Values are Mean $\pm$ S.D.

Test variables	Normal	Obese	p-value
Hb (g/dL)	14.96 $\pm$ 0.85	12.46 $\pm$ 0.94	0.0001
Iron (μmol/L)	22.34 $\pm$ 1.12	16.58 $\pm$ 0.88	0.0001
TIBC (μmol/L)	59.24 $\pm$ 1.89	52.62 $\pm$ 1.24	0.0001
Ferritin (μg/L)	81.62 $\pm$ 1.22	91.06 $\pm$ 1.86	0.0001
Glucose (mg/dL)	96.74 $\pm$ 10.58	100.44 $\pm$ 11.14	0.03, NS
Total Cholesterol (mg/dL)	168.6 $\pm$ 10.9	191.0 $\pm$ 9.86	0.0001
HDL (mg/dL)	41.4 $\pm$ 2.52	36.8 $\pm$ 1.31	0.0001
LDL (mg/dL)	101.4 $\pm$ 3.61	112.4 $\pm$ 3.28	0.0001
Triglyceride (mg/dL)	108.2 $\pm$ 4.82	151.3 $\pm$ 4.22	0.0001
Vit. C (mg/dl)	1.81 $\pm$ 0.34	2.48 $\pm$ 1.42	0.0001
Glutathione (units/mg)	5.38 $\pm$ 0.82	8.42 $\pm$ 0.94	0.0001
SOD (units/mg of Hb)	16.514 $\pm$ 2.25	39.76 $\pm$ 3.24	0.0001

Note: NS: Non-Significant, $p < 0.05$ was is the level of significance.

Table-3: Correlation of mean Body Mass Index with other test variables in obese individuals.

Test variables	Pearson's value(r)	p-value
Hb (g/dl)	-0.72	<0.001
Iron ($\mu\text{mol/L}$)	-0.81	<0.001
TIBC ($\mu\text{mol/L}$)	-0.82	<0.001
Ferritin ($\mu\text{g/L}$)	0.79	<0.001
Glucose (mg/dL)	0.68	<0.001
Vit. C (mg/dL)	0.18	>0.05, NS
Glutathione (units/mg)	0.17	>0.05, NS
SOD (units/mg of Hb)	0.35	<0.001
Total Cholesterol (mg/dL)	0.42	<0.001
HDL (mg/dL)	-0.38	<0.001
LDL (mg/dL)	0.33	<0.001
Triglyceride (mg/dL)	0.36	<0.001

Note: NS: Non-Significant, $p < 0.05$ was is the level of significance.

Table-4: Correlation of mean Ferritin level with other test variables in obese individuals.

Test variables	Pearson’s value(r)	p-value
BMI (kg/m ²)	-0.81	<0.001
Iron (μmol/L)	-0.82	<0.001
TIBC (μmol/L)	-0.79	<0.001
Hb (g/dL)	-0.58	<0.001
Glucose (mg/dL)	0.38	<0.001
Total Cholesterol (mg/dL)	1.23	<0.001
HDL (mg/dL)	-1.62	<0.001
LDL (mg/dL)	1.95	<0.001
Triglyceride (mg/dL)	1.58	<0.001
Vit. C (mg/dL)	0.16	>0.05, NS
Glutathione (mg/dL)	0.39	>0.05, NS
SOD (units/mg of Hb)	0.34	<0.001

Note: NS: Non-Significant, p<0.05 was is the level of significance.

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Figure-1: Flow chart describing the steps involved in establishing the study sample from among 450 participants selected randomly for their inclusion in the study.