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SERUM FERRITIN IS AN EARLY RISK INDICATOR OF INSULIN RESISTANCE IN PREDIABETES

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ABSTRACT

Introduction: Serum ferritin, a marker of iron stores has been implicated to play a role in many acute and chronic disease conditions. Several epidemiological studies have reported excess deposition of iron in tissues related to insulin resistance (IR).

Aim & Objective: The aim of this study is to investigate the risk of iron stores as assessed by serum ferritin to see where it could be an early indicator of insulin resistance in Pre-diabetes.

Material and Methods: This case control study included a total of 224 participants with 112 Pre-Diabetic individuals who attended the OP Department of General Medicine and matchedwith 112 normal healthy individuals who attended the Master health checkup OP in SRM MCH & RC. Their fasting blood samples were collected to estimate FBS, PPBS, Iron, Ferritin and Insulin. Insulin resistance was calculated using homeostasis model assessment insulin resistance (HOMA-IR) formula. Statistical analysis was performed by bivariate correlation and paired samples t test between cases and controlsby SPSS IBM social software.

Results: Serum Ferritin (110.39 ± 13.52), Iron (113.32 ± 14.81), %TS (32.18 ± 6.24), Serum Insulin (17.04 ± 1.89) and HOMA-IR (4.98 ± 0.77) were significantly high in Pre-Diabetics when compared with controls Serum Ferritin (43.46 ± 9.26), Iron (70.02 ± 6.79), %TS (18.79 ± 3.04), Serum Insulin (8.02 ± 1.86) and HOMA-IR (1.71 ± 0.59). Similarly we found a significant positive correlation between Ferritin and Insulin (r = .956**, P = <0.001) in Pre-Diabetics.

Conclusion: Serum ferritin levels were positively associated with serum insulin and HOMA-IR in individuals with Pre-Diabetes, suggesting increasedserum ferritin could be an earlyrisk indicator of IRin Pre-Diabetes.

Keywords: Pre-diabetes, Ferritin, Insulin Resistance (IR), Basal metabolic index (BMI).

INTRODUCTION

Pre-Diabetes is a metabolic disorder characterised by impaired fasting glucose (IFG) or impaired glucose tolerance (IGT) [1].Iron plays an essential role as a cofactor for fuel oxidation and electron transport, but in excess, it was considered to bepotentially toxic under aerobic condition [2]. Iron overload has been shown to initiate formation of reactive oxygen species (ROS) such as superoxide anion ($O_2^{\cdot}$), hydroxyl radical ($OH^{\cdot}$) and through Fenton reaction [3] and thereby induce oxidative stress [4].The ensuing an oxidative stress occurs when there is a distortion in the redox balance of the cell causing damages proteins, DNA and lipid in membrane components which results in mitochondrial dysfunction [5,6]. Studies have shown that the linking glucose homeostasis and iron metabolism is bidirectional, which means that dysglycemia can also affect in iron metabolism [7].Pancreatic iron accumulation is predictor of impaired glucose metabolism in an individual with β thalassemia and hereditary hemochromatosis [8,9]. Excess deposition of iron affects insulin synthesis and secretion in the β cells of pancreas [10,11] and interferes with the insulin-extracting capacity of the liver [12].Insulin resistance is characterized by a diminished ability of cells or tissues to respond to physiological levels of insulin [13]. Genetic and environmental factors, including aging, obesity, lack of exercise, and stress, contribute to insulin resistance [14]. Disorders of glucose and lipid metabolism cause defects in insulin signaling that are linked to various pathological conditions [15),Serum Ferritin is a protein that stores iron and releases it in regulating iron homeostasisto bind iron atoms thus preventing any toxic cellular damage by reactive oxygen species (ROS) [16] and it is also a positive acute reactant that has been shown increased

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in various acute and chronic disease conditions [17,18].In this study, we made an attempt to investigate the risk of iron stores as assessed by serum ferritin to see where it could be an earlyindicator of insulin resistance in Pre-diabetes.

Materials and Methods

This is a case control study carried out in a group of individuals aged 25 to 45 years who attendedthe OP department of general medicine and master health checkup at SRM Medical College Hospital and Research Centre, Chennai, Tamil Nadu, India.

Sample size calculation: $n=Z^2P(1-P)/E^2$

Group-I = 112 Normal Healthy Controls

Group-II = 112 Pre-Diabetic individuals

The study protocol was approved by the institutional ethical committee (ECN: 1400/ICE/2018)and informed written consent was obtained from all the study participant**Inclusion Criteria**

The Pre-Diabeticindividuals (both males and females) were diagnosed on the basis of impaired fasting plasma glucose and HbA1c levels.

Group – I (Healthy Control): Individuals with Fasting plasma glucose levels 70 to 109 mg/dl and HbA1c 5.0 to 5.6%,were considered as healthy controls with no clinical evidence of acute and chronic diseases.

Group – II (Pre-Diabetics): Individuals with impaired fasting glucose levels between 110 to 125 mg/dland HbA1c levels 5.7 to 6.4 % were diagnosed as prediabetes individuals.

Exclusion Criteria

The study excluded participants diagnosed to have Type 1 DM, Gestational Diabetes Mellitus and those who had anaemia, bleeding piles, iron therapy, hepatic disease, acute and chronic infective disease.

Anthropometric measurement

Height (meters) and Weight (kg) was measured by the physician during the clinical examination, using a stadiometer, electronic digital weighing scale and with the subject wearing light clothing. Body mass index (BMI) was calculated based on weight divided by the height squared formula[19].

Biochemical measurements

5ml of venous blood was collected from all the study participants after an overnight 12hours fasting.FBS estimated by GOD-POD method, HbA1c by HPLC, Ferritin by Latex agglutination reaction in Beckman Coulter and Insulin by Electro Chemi Luminescence technique, Lipid profile that include Total cholesterol by Cholesterol Oxidase method, Triglycerides by Glycerol peroxidase method & HDL-C, LDL-C was estimated by Direct methodusing Beckman Coulter Auto analyser (AU480).

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Statistical Analysis

All the data were analysed using Statistical Package for Scientific Studies (SPSS) version 21. The results were denoted as Mean ± Standard Deviation. Paired t-test was used to evaluate the variance between the mean levels of various parameters. Correlation between various variables was assessed using Bivariate Pearson's correlation equation between Ferritin and other study variables. The p-value <0.05 was considered significant.

Results

Table 1: A sample of 224 control (male 56, female 56) and Pre-Diabetes (male 56, female 56) chosen from SRM Medical hospital OP department of general medicine were evaluated in terms of anthropometric and biochemical parameters. With the purpose of assessing the Hb, iron, ferritin status in this population, Plasma Hemoglobin, Serum Iron and Serum ferritin levels were high in males compared with females.

Table 2: A sample of 224 age, sex matched control 112 (both Male, Female) and pre-Diabetes 112 (both Males, Females) chosen from SRM Hospital OP department of general medicine were evaluated in terms of anthropometric and biochemical parameters. With the purpose of assessing the fasting glucose, post prandial glucose, HbA1c, lipid profile, Hemoglobin, Iron, TIBC, %TS, Ferritin, Insulin and HOMA-IR status in this population. Serum Ferritin and Insulin levels were highly significant (P = < 0.001)when compared between cases and controls.

Table 3 shows that overall correlation of Ferritin with other biochemical variables. Serum ferritin highly significant with fating glucose (r = .959**, P = < 0.001), PPBS (r = .947**, P = < 0.001), Total Cholesterol (r = .921**, P = < 0.001), LDL-C (r = .911**, P = < 0.001), Iron (r = .957**, P = < 0.001), % TS (r = .904**, P = < 0.001), serum Insulin (r = .956**, P = < 0.001) and HOMA-IR (r = .943**, P = < 0.001). Serum ferritin was negatively correlated with HDL-C (r =-.869**, P = 0.001) and TIBC (r = -.572**, P = 0.001).

Table 1: Demographic and Biochemical variables of the participants in the study groups.

Parameter	Control		Pre-Diabetes	
	Male (n = 56)	Female (n = 56)	Male (n = 56)	Female (n = 56)
Age (Years)	36.75 ± 6.19	35.57 ± 6.59	37.04 ± 6.41	36.79 ± 6.199
BMI ((kg/m2)	21.46 ± 2.11	21.21 ± 1.85	28.00 ± 2.11	27.86 ± 1.67
FBS (mg/dl)	88.50 ± 3.95	86.86 ± 5.31	119.00 ± 3.88	116.82 ± 4.81
PPBS (mg/dl)	99.46 ± 5.30	97.75 ± 6.19	139.50 ± 8.15	137.64 ± 7.93
HbA1c (%)	5.43 ± 0.49	5.39 ± 0.49	6.46 ± 0.50	6.32 ± 0.47
Total cholesterol (mg/dl)	190.71 ± 12.69	175.50 ± 10.85	235.96 ± 15.89	224.57 ± 14.85
Triglycerides (md/dl)	120.75 ± 23.39	104.61 ± 3.38	191.68 ± 42.22	183.96 ± 34.64
HDL-C (mg/dl)	47.79 ± 3.82	52.50 ± 4.06	40.07 ± 2.64	41.71 ± 2.77
LDL-C (mg/dl)	109.07 ± 8.13	98.93 ± 5.19	153.96 ± 17.32	151.96 ± 17.12
Hb (g/dl)	15.07 ± 1.00	12.50 ± 0.63	15.36 ± 0.81	12.89 ± 0.67
Iron(µg/dl)	75.00 ± 4.78	65.04 ± 4.42	120.25 ± 12.94	106.39 ± 13.32
TIBC(µg/dl)	365.04 ± 25.41	387.18 ± 12.70	352.00 ± 24.25	360.00 ± 20.56
Ferritin(ηg/ml)	51.25 ± 5.86	35.68 ± 3.92	122.93 ± 4.61	97.86 ± 5.29
Insulin(µIU/ml)	8.57 ± 1.81	7.46 ± 1.75	17.64 ± 1.75	16.43 ± 1.85
HOMA-IR	1.86 ± 0.64	1.57 ± 0.49	5.14 ± 0.74	4.82 ± 0.76

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Table 2: Demographic and Biochemical variables of the participants in the control and Pre-diabetes.

Parameter	**Group 1 Control (n = 112)**	**Group 2 Pre-Diabetes (n = 112)**	**P Value**
Age (years)	36.16 ± 6.39	36.91 ± 6.28	0.003
BMI (kg/m2)	21.34 ± 1.98	27.93 ± 1.91	0.001
Fasting Glucose (mg/dl)	87.68 ± 4.73	117.91 ± 4.49	0.001
PPBS (mg/dl)	98.61 ± 5.80	138.57 ± 8.06	0.001
HbA1c (%)	5.41 ± 0.49	6.39 ± 0.49	0.005
Total cholesterol (mg/dl)	183.11 ± 14.02	230.27 ± 16.34	0.001
Triglycerides (mg/dl)	112.68 ± 18.50	187.82 ± 38.64	0.004
HDL-C (mg/dl)	50.14 ± 4.58	40.89 ± 2.82	0.005
LDL-C (mg/dl)	104.00 ± 8.49	152.96± 17.17	0.001
Hb (g/dl)	13.79 ± 1.53	14.13 ± 1.44	0.05
Iron (µg/dl)	70.02 ± 6.79	113.32 ± 14.81	0.005
TIBC (µg/dl)	376.11 ± 22.88	356.00 ± 22.74	0.006
% TS	18.79 ± 3.04	32.18 ± 6.24	0.001
Ferritin (ηg/ml)	43.46 ± 9.26	110.39 ± 13.52	< 0.001
Insulin (µIU/ml)	8.02 ± 1.86	17.04 ± 1.89	< 0.001
HOMA-IR	1.71 ± 0.59	4.98 ± 0.77	< 0.001

Values represent Mean ± SD between two groups. P value <0.05 was considered significant.

Table 3: Overall Correlation analysis of Ferritin with other biochemical variables.

Parameter	**Ferritin (ηg/ml)**	
	r value	**P Value**
Age (years)	.198*	0.003
BMI (kg/m2)	.888**	0.001
Fasting Glucose (mg/dl)	.959**	< 0.001
PPBS (mg/dl)	.947**	< 0.001
HbA1c (%)	.772**	0.001
Total cholesterol (mg/dl)	.921**	< 0.001
Triglycerides (mg/dl)	.838**	0.001
HDL-C (mg/dl)	-.869**	0.001
LDL-C (mg/dl)	.911**	< 0.001
Hb (g/dl)	.413**	0.005
Iron (µg/dl)	.957**	< 0.001
TIBC (µg/dl)	-.572**	0.006
%TS	.904**	< 0.001
Insulin (µIU/ml)	.956**	< 0.001
HOMA-IR	.943**	< 0.001

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DISCUSSION:

Many diseases are associated with iron overload or iron deficiency. Although iron plays an essential role as a cofactor for fuel oxidation and electron transport, but in excess, it was considered to be potentially toxic under aerobic condition. Iron is a transition metal capable of causing oxidativetissue damage by catalyzing the formationof reactive oxygen species (ROS) such as superoxide anion ($O_2^{\cdot}$), hydroxyl radical ($OH^{\cdot}$) and through Fenton reaction [20].Iron overload can indicate to insulin resistancethrough mechanisms related to both reducedextraction of insulin and impairedinsulin secretion [21].Fernandez-Real JM suggested that iron deposition in pancreatic β-cells may also impair insulin secretionin more advanced states of ironoverload [22]. Serum ferritin is a widely used markerof iron status and an acute phase reactant in epidemiological studiesmay be increased in the presence of inflammation [23,24].Sichuanwas reported, ferritin concentrations were significantly elevated insubjects with diabetes compared to those withnormal glucose tolerance in both men and women [25].C E Wrede was suggested thatferritin is positively correlated with elements of the insulin resistancesyndrome in a representative German population [26]. We observed similar findings in our study in that Ferritin levels were positively associated with insulin, FBS, PPBS, Total cholesterol, LDL - C, Iron, % Transferrin saturation and HOMA-IR. Ferritin was negatively associated with HDL– C and TIBC. It appear that the biological implication for the association between serum ferritin and insulin resistance needs to be studied in depth in future investigations.

CONCLUSION:

Our study is unique in that we observed an association of serum ferritin and insulin resistance in individuals with pre-Diabetes and this imply that serum ferritin could serve as an early risk biomarker to be considered in lifestyle intervention programme.

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