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Original Article

Physiology Section

Association of Serum Iron Indices with Insulin Resistance Index in Euglycaemic Offspring's of Diabetic and Non Diabetic Parents: A Case-control Study

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ABSTRACT

Introduction: Altered iron metabolism may be a risk factor for various diseases. There are studies showing that altered iron metabolism is frequently found in type 2 diabetes mellitus patients, but there is lacunae of literature in offspring's of diabetic parents.

Aim: To compare serum iron indices in euglycaemic offsprings of diabetic and non diabetic parents and also to correlate serum iron indices with HOMA-IR.

Materials and Methods: This was a case-control study conducted in RL Jalappa Hospital and Research Centre, Kolar, Karnataka, India, from February 2019 to January 2020. Total 80 euglycaemic healthy individuals were recruited. Out of 80 subjects 40 were cases and 40 were controls. Offspring of non diabetic parents as control group and offspring of diabetic parents as a case group were included in the study. Anthropometric measurements like weight, height was measured and Body Mass Index (BMI) was calculated. Laboratory parameters like complete haemogram, Fasting Blood Sugar (FBS), serum iron markers like serum iron, serum ferritin, serum Total Iron Binding Capacity (TIBC), serum insulin and high sensitive C-reactive protein were analysed and compared between cases and controls. Homeostasis Model Assessment-estimated Insulin Resistance (HOMA-IR) was calculated.

Results: Mean age of cases was 34.65±3.06 years and controls was 35.75±3.43 years respectively. Out of total 24 were females and 56 were males. Serum iron markers like serum iron and serum ferritin was significantly increased and serum TIBC was significantly decreased among euglycaemic offspring's of diabetic parents compared to offspring's of non diabetic parents. Serum ferritin (r-value=0.389, p-value=0.007) and serum iron (r-value=0.483, p-value=0.001), showed positive correlation with HOMA-IR among euglycaemic offspring's of diabetic parents. On multiple regression analysis, in model 1 (HOMA-IR as dependent variable and serum ferritin, serum iron and serum TIBC as independent variable) serum iron emerged as a significant predictor of insulin resistance, whereas after adding confounding variables like BMI, high sensitivity C-Reactive Protein (hsCRP), lipid profile with serum iron markers in model 2, serum iron emerged as independent predictor of insulin resistance though regression equations was not statistically significant.

Conclusion: Offsprings of diabetic parents showed iron overload and insulin resistance which predisposes them to develop T2DM and serum iron can be used as one of the marker of insulin resistance among offspring of diabetic parents along with other parameters.

Keywords: Diabetes mellitus, Iron overload, Lipid profile, Serum ferritin

INTRODUCTION

The prevalence of Type 2 Diabetes Mellitus (T2DM) is increasing worldwide and India once known "diabetes capital of the world" was home to 61.3 million patients with T2DM in 2011 with predictions of 101.2 million diabetics by 2030. India is second only to China which is home to 92.3 million diabetics [1]. T2DM is a complex, heterogeneous and polygenic metabolic disease condition characterised by insulin resistance and insulin secretary defect. Its pathogenesis appears to involve complex interactions between genetic and environmental factors [2]. The prevalence of DM among the offspring with one diabetic parent was 40% which increases to 80% if both the parents are diabetic. Offsprings with the strong family history, the age of onset of diabetes is known to be much earlier than their parents [3].

Obesity and family history are the major risk factors of T2DM [4]. Apart from this excess iron also contributes to the development of T2DM [5]. Iron was a transition metal and a potential catalyst in cellular reaction that produces reactive oxygen species. Iron and insulin show bidirectional relationship in the body. Insulin influences the iron uptake and storage by increasing the cell surface transferrin receptors, reciprocally iron influences the insulin activity by interfering with glucose uptake and utilisation. Iron causes hyperinsulinaemia by decreasing the insulin uptake and metabolism by hepatocytes leading to hepatic insulin resistance and subsequently to decreased insulin secretion and then to the development of T2DM [6]. Studies have shown that high ferritin levels even within the physiological limits also influences the development of T2DM [2,7].

Studies done among T2DM patients demonstrated that elevated serum ferritin and transferrin receptors are associated with increased risk of T2DM and risk decreases by reducing the body iron stores [2,8,9]. Some of the studies also concluded that, serum ferritin may serve as surrogate indicator of glycaemic status in T2DM patients [1,2,10]. A study done in offsprings of diabetic parents revealed significant differences in serum iron indices between offspring with diabetic parents compared to their counterparts and serum iron indices are strongly correlated with insulin resistance thus stating that excess iron and insulin resistance exist in non diabetic offsprings of diabetic parents that predispose them to develop T2DM [11].

A study proposed that the abnormalities in iron metabolism in offspring of T2DM represent an additional component of metabolic syndrome or insulin resistance syndrome. They showed that these abnormalities are present even before the change in glucose metabolism becomes apparent [12]. According to the studies, it was seen that excess serum iron indices was an potential risk

factor for T2DM along with other risk factors like obesity, age and family history and most of the studies were done in diabetic and prediabetic patients [2,8,13].

Thus, there was a need to compare serum iron indices among euglycaemic offspring's of diabetic parents with offspring's of non diabetic parents and associate it with insulin resistance index. Hence, the present study was conducted to compare serum iron indices in euglycaemic offsprings of diabetic and non diabetic parents and to correlate the serum iron indices with HOMA-IR.

MATERIALS AND METHODS

This was a case-control study conducted in RL Jalappa Hospital and Research Centre, Kolar, Karnataka, India, from February 2019 to January 2020. The ethical clearance was obtained from Central Ethical Committee approval (IEC no:Project:SDUAHER/Res.proj/123/2016-17). The protocol was explained to the subjects and written informed consent was obtained.

Inclusion criteria: All healthy subjects in the age group of (25-40 years) comprising of both genders and offsprings of diabetic parents sharing at least 50% genetic relation with their probands were included as cases. Controls were healthy, age and sex matched offsprings of non diabetic parents were included in this study.

Exclusion criteria: Subjects were screened clinically and if were found to be suffering with any acute or chronic medical, psychiatric conditions and on any medications were excluded from the study. All the female subjects with irregular menstrual cycle were also excluded.

Sample size calculation: Sample size was estimated by using the mean ferritin levels in adults aged 25-40 years born to diabetic parents as 98.3±57.7 ng/dL and those born to non diabetic parents as 62.0±41.2 ng/dL at 95% confidence limit and 90% power sample size of 41 was obtained in each group. With 10% non response sample size of 41+3=44 subjects was included in each group [12]. Sample size collected was 88, out of which four of them had haemoglobin <10 gm/dL, three of them had FBS >100 mg/dL as per the inclusion criteria only euglycaemic offsprings will be included and one drop out after demographic data collection, so could not collect blood sample for biochemical parameters estimation. Hence, final sample size was 80.

Systematic probability random sampling was done to select the subjects. Every 2nd diabetic or nondiabetic parent's offsprings attending Medicine Outpatient Department was recruited.

Total 80 subjects were divided into two groups:

- Cases- 40 euglycaemic offspring's of diabetic patients.
- Controls- 40 offspring's of non diabetic patients.

Study Procedure

Data collection: Demographic details like age, family history of T2DM of the cases and controls were collected. Anthropometric parameters like weight, height was measured. Body Mass Index (BMI) was calculated and matched between euglycaemic offspring's of diabetic and non diabetic offspring's. Weight in kilograms was measured using standard calibrated balance scale with sensitivity to the nearest 0.1 kg (Omron HN 286 Ultra-Thin Automatic Personal Digital Weight Scale with large Liquid Crystal Display (LCD) display and 4 Sensor Technology for Accurate Weight Measurement). Height in centimeters was obtained using stadiometer (Indo-Surgicals Height Measuring Scale Stadiometer Measurement Tape) and BMI was calculated using formula weight in kg/height^2 in m square [14].

Biochemical parameters: Subjects were instructed to report to the lab after 12 hours of overnight fast. Venous blood sample of 8 mL was drawn in sitting posture for estimation of haemoglobin, high-sensitivity C-Reactive Protein (hsCRP), serum ferritin, serum iron, serum. Total Iron Binding Capacity (TIBC), serum insulin and lipid parameters [Table/Fig-1] [15-24].

Parameter	Test	Normal range
Fasting blood sugar [15]	Glucose oxidase method	74-100 mg/dL
Total cholesterol (TC) [16]	Cholesterol oxidase peroxidase method	120-200 mg/dL
Triglyceride (TG) [17]	Lipase glycerol kinase peroxidase method	44-150 mg/dL
High density cholesterol (HDL) [18]	Direct precipitation method	40-60 mg/dL
Low density cholesterol [19]	Friedwalds formula	120-246 mg/dl
Serum iron [20]	Pyridyl Azodye dry chemistry	Female: 37-170 µg/dL Males: 49-181 µg/dL
Serum total iron binding capacity [21]	Colorimetric chromazurol dye binding method	Males: 261-462 µg/dL Females: 265-497 µg/dL
Serum ferritin [22]	Enhanced Chemiluminesence method	Males: 17.9-464 mg/mL Females: 6.24-137 mg/mL
Serum insulin [23]	Enzyme linked immunosorbent assay method	2.0-25.0 µU/mL
Homeostasis model assessment for insulin resistance [24]	HOMA-IR index=Fasting insulin (micro/L)* Fasting glucose (nmol/L)/22.5	2-2.5

[Table/Fig-1]: Normal reference range for all the biochemical parameters assessed in the present study [15-24].

STATISTICAL ANALYSIS

Data was entered into Microsoft excel data sheet and was analysed using Statistical Package for Social Sciences (SPSS) software version 22.0. The normality of variables was evaluated by the Kolmogorov-Smirnov test. Continuous data was represented as mean and standard deviation. Comparisons were done between the two groups using independent t test. Pearson's correlation was done to correlate serum iron indices and HOMA-IR index among euglycaemic offspring's of diabetic parents. To examine the association between HOMA-IR and other variables, multiple linear regression analyses were tested in euglycaemic offspring's of diabetic parents. The p-value <0.05 was considered as statistically significant.

RESULTS

The study included 40 euglycaemic offspring's of diabetic patients and 40 offspring's of non diabetic patients with mean age of 34.65±3.060 years and 35.75±3.43 years, respectively. Out of 80 subjects 56 were males and 24 were females.

A Kolmogorov-Smirnov and a visual inspection of their histograms, normal Q-Q plots and box plots showed that the HOMA-IR values were normally distributed for both cases and controls with a skewness of 0.356 (SE=0.374) and a kurtosis of 0.374 (SE=0.733) for cases (p-value <0.2) and skewness of 1.055 (SE=0.374) and kurtosis of 1.841 (SE=0.733) for controls (p-value <0.129) [Table/Fig-2].

Groups	Skewness (SE)	Kurtosis (SE)	Statistics	df	Significance
Cases	0.356 (SE=0.374)	0.374 (SE=0.733)	0.099	40	0.2
Control	1.055 (SE=0.374)	1.841 (SE=0.733)	0.123	40	0.129

[Table/Fig-2]: Kolmogorov-Smirnov test for normality of dependent variable (HOMA-IR). df: Degree of freedom; p-value <0.05 was considered as statistically significant

Age, BMI, haemoglobin, FBS was matched between the groups i.e, offspring's of diabetic and non diabetic parents [Table/Fig-3]. There was a statistically significant increase in mean serum insulin

(p-value=0.001), high sensitive C-reactive protein (p-value=0.006) and HOMA-IR (p-value=0.001) among the cases as compared to controls.

Parameters	Cases (Mean±SD)	Controls (Mean±SD)	p-value
Age (years)	34.65±3.060	35.75±3.43	0.143
Weight (kg)	68.28±11.60	68.30±13.3	0.993
Body mass index (kg/m^2)	25.60±3.10	25.23±3.91	0.639
Haemoglobin (gm/dL)	14.84±1.62	14.16±1.63	0.066
Fasting blood sugar (mg/dL)	88.45±5.2	87.58±6.1	0.495
High sensitive C-reactive	2.85±1.29	1.94±1.5	0.006*
Serum insulin (micro/L)	16.7±6.3	9.2±5.6	0.001**
HOMA-IR	2.89±1.39	1.72±1.05	0.001**

[Table/Fig-3]: Comparing demographic, anthropometric and metabolic parameters between cases and controls.
Independent t-test to compare the cases and controls; HOMA-IR: Homeostatic model assessment-insulin resistance; p-value <0.05 was considered as statistically significant

The [Table/Fig-4] depicts comparison of serum iron parameters between cases and controls. There was a significant increase in mean of serum ferritin (p-value=0.001), serum iron (p-value=0.001) among the cases as compared to controls. There was significant decrease in mean of serum total iron binding (p-value=0.002) among cases as compared to controls.

Parameters	Cases (Mean±SD)	Controls (Mean±SD)	p-value
Serum ferritin (ng/mL)	91.43±52.8	45.72±37.8	0.001
Serum total iron binding (mcg/dL)	214.7±84.18	290.8±127.5	0.002
Serum iron (mcg/dL)	212.7±59.5	148.4±58.8	0.001

[Table/Fig-4]: Independent t-test for comparing serum iron parameters between cases and controls.
p-value <0.05 was considered as statistically significant

The [Table/Fig-5] shows that there was significant moderate positive correlation between serum ferritin (r=0.389) and serum iron (r=0.483) with HOMA-IR in offsprings of T2DM. Serum TIBC shows weak negative correlation with HOMA-IR (r=-0.246) in cases, but statistically not significant.

Parameter	HOMA-IR index	
	r-value	p-value
Serum ferritin (ng/mL)	0.389	0.007
Serum TIBC (mcg/dL)	-0.246	0.063
Serum iron (mcg/dL)	0.483	0.001

[Table/Fig-5]: Pearson's correlation between serum iron indices and HOMA-IR index among cases.
TIBC: Total Iron binding capacity; p-value <0.05 was considered as statistically significant

The [Table/Fig-6] shows there was significant increase in mean values of in serum cholesterol (p-value=0.015), serum triglycerides (p-value=0.002), Low Density Lipoprotein (LDL) (p-value=0.001) in cases compared to controls. The other lipid parameters High Density Lipoprotein (HDL), HDL/LDL showed significant decrease of mean values in cases compared to controls.

Parameters	Cases (Mean±SD	Control (Mean±SD)	p-value
Serum cholesterol (mg/dL)	143.50±51.2	119.8±31.4	0.015
Serum triglycerides (mg/dL)	187.6±84.3	139.2±48.2	0.002
High density lipoprotein (HDL) mg/dL	38.05±5.6	42.88±7.21	0.001
Low density lipoprotein (LDL) mg/dL	103.5±40.4	58.00±33.6	0.001
HDL/LDL	0.568±0.42	0.891±0.45	0.001

[Table/Fig-6]: Independent t-test comparing serum lipid parameters in cases and controls.
p-value <0.05 was considered as statistically significant

The [Table/Fig-7] depicts the association of HOMA-IR with serum iron indices and confounding factors. In Model 1 offsprings of T2DM parents predicted [HOMA-IR]=0.468+0.007 (serum ferritin)-0.001 (Serum TIBC)+0.009 (Serum iron) offsprings of T2DM parents. HOMA-IR increased by index for each ng/mL of serum ferritin, decreased by 0.001 index for each mcg/dL of serum TIBC and increased by 0.005 index for each mcg/dL of serum Iron. Thus, serum iron was significant predictor of HOMA-IR in model 1.

HOMA-IR	Unstandardised β	SE	Standardised β	p-value	R^2
Model 1	Constant:0.468, F (df:3,36)=4.803, p-value <0.006				
Serum ferritin	0.007	0.004	0.248	0.113	0.226
Serum TIBC	-0.001	0.003	-0.013	0.905	
Serum iron	0.009	0.004	0.382	0.040	
Model 2	Constant:-3.691, F (df:9,30)=1.981, p-value <0.078				
Serum ferritin	0.004	0.005	0.166	0.359	0.185
Serum TIBC	-0.001	0.004	0.057	0.774	
Serum iron	0.011	0.005	0.463	0.025	
HSCRP	0.033	0.167	0.031	0.845	
Serum cholesterol	0.002	0.005	0.071	0.670	
Serum triglycerides	0.002	0.003	0.100	0.544	
HDL	-0.007	0.048	-0.027	0.888	
LDL	0.003	0.006	0.074	0.683	
BMI (Kg/m^2)	0.133	0.073	0.295	0.078	

[Table/Fig-7]: Multiple linear regression analyses for HOMA-IR (stepwise method) in cases.
HDL: High density lipoprotein; LDL: Low density lipoprotein, TIBC: Total iron binding capacity; HSCRP: High senstive C-reactive protein; BMI: Body mass index

In model 2, HSCRP, BMI, serum cholesterol, serum triglycerides, serum HDL, serum LDL was added with serum iron indices. A regression equation was found {F (df:9,30)=1.981, p-value <0.078}, with an R^2 of 0.185 for offsprings of T2DM parents which was not statistically significant. Serum iron emerged as an independent significant predictor in offspring's of T2DM parents even after adjusting for all the covariates (HSCRP, BMI, serum cholesterol, serum triglycerides, HDL, LDL).

DISCUSSION

The present study showed significant increase in insulin resistance and serum insulin levels (p-value <0.001) in euglycaemic offspring of T2DM parents compared to euglycaemic offsprings of non diabetic parents. Individuals with positive family history of diabetes are at 3-4 fold high risk of developing diabetes compared to negative family history diabetes [25].

The present study corroborates with the findings of other studies that serum insulin levels and insulin resistance as assessed by HOMA-IR was increased in euglycaemic offspring of diabetic parents [9,26]. In the present study, as per the inclusion criteria after matching for age, BMI, haemoglobin level and FBS parameters the serum ferritin and serum iron were significantly increased (p-value <0.001) and serum TIBC was decreased in euglycaemic offspring's of T2DM parents, though the glucose metabolic parameters were in normal range. The serum iron indices were in normal range in euglycaemic offspring's of diabetic parents but higher when compared to euglycaemic offspring's of non diabetic parents. Thus, indicating changes in serum iron indices precedes changes in glucose level among the euglycaemic offspring's of T2DM [9,26]. The results of this study further illustrated that there was significant positive correlation between HOMA-IR with serum iron and serum ferritin in euglycaemic offspring's T2DM diabetic parents. Several studies showed normoglycaemic offspring's with family history of T2DM had significant increase in serum iron indices compared to control group [27-29].

In model 1 and model 2 after adjusting for the confounding variables (serum iron indices, lipid parameters, inflammatory marker HSCRP and BMI) multiple linear regression analysis showed that elevated serum iron levels emerged as a significant independent predictor of HOMA-IR in glycaemic offspring's of T2DM parents (model-1 p-value <0.040 and model-2 p-value <0.025). A study done among T2DM patients showed no association between serum ferritin and HOMA-IR but there was association with transferrin saturation [10]. Several studies have shown association between serum ferritin and insulin resistance in diabetics in elderly Chinese population [30,31]. A cross-sectional survey of 8,235 participants in china reported serum ferritin levels were associated with higher risks of diabetes, as increase in serum ferritin level causes increase in the levels of HbA1c, and HOMA-IR [32]. In offspring's of T2DM serum iron indices elevation might be due to elevation in insulin resistance in liver.

This study also showed significant increase (p-value <0.05) in serum iron indices and along with metabolic syndrome components such as lipid parameters and serum insulin in offspring's of T2DM parents, thus indicating that serum iron and insulin resistance coexist with glycaemic offspring's of T2DM parents which was also supported by the positive correlation between these two. The observation was corroborating with findings that serum iron elevation and insulin resistance coexist with offspring's of diabetic patients that predispose them to develop T2DM [11].

The exact mechanism of the association between serum iron and diabetic disorder yet to be determined. Few possible biological pathways may be proposed to describe the observed findings. Firstly, iron is regarded a catalyst in the production of highly reactive oxygen radicals and excess body iron may be involved in insulin signaling [33,34]. Secondly, iron causes peroxidation of free fatty acids, leading to accelerated production of free radicals, which further leads to decrease glucose uptake in the muscles and increased glucose production in the liver causing development of insulin resistance. Lastly, a study showed relationship between serum iron and inflammation which is a predisposing risk factor for insulin resistance in offspring's of T2DM and also the elevated iron level could impair the function of pancreatic β cells in humans [35,36]. Results of this study are consistent with the above research showing significant increase in high sensitive C-reactive protein (p-value <0.05) among the euglycaemic offspring's of T2DM. Hepcidin a peptide synthesised in the liver, is elevated in response to hypoxia or inflammation and is correlated with increases in ferritin, where leptin induces the expression of hepcidin via the JAK2/STAT3 pathway [29,30,37].

Limitation(s)

Study was cross-sectional in nature and causal relationship between serum iron markers and insulin resistance must be determined by longitudinal studies. History of dietary intake of iron has not been elicited as this also might have increased serum iron levels in euglycaemic offspring's of T2DM parents.

CONCLUSION(S)

This study concluded that several markers of iron metabolism are increased in offspring of diabetic parents and also showed positive correlation with HOMA-IR. Serum iron emerged as an independent significant predictor of HOMA-IR. These findings suggest that iron metabolism related factors may contribute to the induction of IR early in the pathogenesis of T2DM. Early identification of the high risk individuals even before they show changes in glucose metabolism with regular screening, the onset of T2DM can be delayed and also serum iron may be included as a one of the component of insulin resistance syndrome.

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