

Association between Iron Indices and Dyslipidemia among Patients with Iron Deficiency Anaemia: A Cross-sectional Study

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ABSTRACT

Introduction: Iron Deficiency Anaemia (IDA) is a major public health problem in India. Iron plays a role in hepatic lipogenesis, being an integral part of some enzymes and transporters involved in lipid metabolism. Since information on the association between iron metabolism and dyslipidemia in adults is limited, it is important to assess the lipid status in iron-deficient patients.

Aim: To study the association of iron indices with the lipid profile among patients with iron deficiency anaemia.

Materials and Methods: This hospital-based cross-sectional study was conducted at the Department of Paramedical and Health Sciences, Parul Institute of Paramedical and Health Sciences, Faculty of Medicine, Parul University, Vadodara, Gujarat, India, from December 2022 to May 2023. In the present study, 100 IDA patients aged between 25-45 years were included. All the participants underwent different tests including Total Cholesterol (TC), Triglyceride (TG), High Density Lipoprotein-Cholesterol (HDL-C), and Low Density Lipoprotein-

Cholesterol (LDL-C). Furthermore, the association between IDA patients and lipid levels was analysed. The data were statistically analysed using Pearson's correlation test and Chi-square test.

Results: In the present study, out of a total of 100 patients, 38 were males and 62 were females. The majority of IDA patients had lower levels of TC, TG, and LDL-C. Low levels of TC and LDL were found in 52 (83.87%) of females and 30 (78.94%) of males. A total of 28 (73.68%) of males and 52 (83.87%) of females had lower values for TG levels. In contrast to TC and TG, a substantial correlation between Haemoglobin (Hb) levels and HDL-C and LDL-C was found. However, Hb had a positive association with TG, HDL-C, and LDL-C and a negative correlation with TC. These results also suggest a substantial correlation between Serum Iron (SI) and TC, TG, and HDL-C, but not a significant correlation with LDL-C.

Conclusion: When observed for dyslipidemia, IDA patients showed significantly lower levels of TC, TG, and HDL-C with respect to their SI levels.

Keywords: Folic acid, Haemoglobin, Iron metabolism, Lipid profile

INTRODUCTION

The most common cause of anaemia worldwide is iron deficiency [1]. IDA remains a major public health problem in India as well [1]. According to the World Health Organisation (WHO), IDA is usually characterised by Hb levels below 13.0 g/dL in adult men and 12.0 g/dL in non pregnant women [2].

As iron is an integral part of Hb, the maximum amount of iron is present in Hb, but it is also stored in the liver in the cores of ferritin shells. Transferrin is the main protein in the blood that binds to iron and transports it throughout the body [1]. Total Iron-Binding Capacity (TIBC) is an essential test used to identify IDA and other diseases of iron metabolism. The ability of transferrin to bind with iron is referred to as its iron binding capability. There are two forms of iron binding capacity: TIBC and Unsaturated Iron Binding Capacity (UIBC). Transferrin levels rise in the blood when iron reserves are reduced. Because just one-third of transferrin is saturated with iron, serum-bound transferrin has an additional 67% binding capability. SI and UIBC are added together to form TIBC. By dividing SI by TIBC and multiplying the result by 100, one can determine the percentage of transferrin saturation [3]. IDA occurs when the body does not get enough iron. Iron helps in forming Red Blood Cells (RBC) [1].

Dyslipidemia refers to a wide variety of genetic and acquired disorders that affect blood lipid levels and contribute greatly to the global burden of cardiovascular disease [4]. Dyslipidemia is a condition in which the bloodstream contains abnormal fats called lipids [5]. Dyslipidemia is classically characterised by abnormal levels of serum cholesterol, TGs, or both, accompanied

by abnormal levels of related lipoprotein species [6]. Cholesterol is transported in the blood by lipoproteins. The main types of lipoproteins are High-density Lipoprotein (HDL) and Low-density Lipoprotein (LDL) [7].

The liver is a key organ for the link between iron metabolism and dyslipidemia [8]. Most studies support the hypothesis that iron plays a role in hepatic lipogenesis, although conflicting outcomes have also been cited [9,10]. Iron is an integral part of some enzymes and transporters involved in lipid metabolism and, as such, may exert a direct effect on hepatic lipid load, intrahepatic metabolic pathways, and hepatic lipid secretion. Several studies have reported an association of ferritin and transferrin with lipid metabolism. Furthermore, data indicate that higher hepatic iron stores were associated with higher liver fat content [11-13]. Some findings suggest that iron in its ferrous form may indirectly affect lipid metabolism by inducing oxidative stress and inflammation [14]. A previous study conducted on diabetic rats showed that low levels of dietary iron reduce the levels of serum TGs, Hb, and cholesterol [15].

Since information on the association between iron metabolism and dyslipidemia in adults is limited, and few studies assert that iron uptake has been shown to be helpful in enhancing lipid status in anaemic patients [16,17]. Furthermore, numerous studies have demonstrated that iron may be a risk factor for the progression of hyperlipidemia. Their examination can help further to rule out the initiation and progression of atherosclerosis along with the subsequent adverse cardiovascular outcomes in the future [18-

21]. However, it is important to note that certain findings indicate contradicting results, suggesting that iron deficiency has no effect on the lipid profile [22].

Hence, the present research was conducted to assess the association of iron profile with dyslipidemia among patients diagnosed with IDA by analysing CBC, SI, Serum Ferritin (SF), TIBC, and lipid profile (TC, TG, HDL-C, LDL-C) tests.

MATERIALS AND METHODS

The hospital-based cross-sectional study was conducted at the Department of Paramedical and Health Sciences, Parul Institute of Paramedical and Health Sciences, Faculty of Medicine, Parul University, Vadodara, Gujarat, India, from December 2022 to May 2023, spanning a six-month period. The study was approved by the Institutional Ethics Committee (IEC no: PUIECHR/PIMSR/00/081734/5312). The study subjects were individually counseled about the study, and an informed consent form was obtained from each patient.

Inclusion criteria: The study included patients aged between 25 and 45 years who had been clinically and biochemically diagnosed with IDA, both males and females. Participants were considered anaemic if Hb <12.0 g/dL, and SI <65 µg/L, SF <10 ng/mL, and TIBC ≥450 µg/dL, as defined by IDA [23].

Exclusion criteria: Patients under 25 and over 45 years of age, those with myocardial infarction, Human Immunodeficiency Virus (HIV) positive individuals, smokers, surgery patients, and pregnant women were excluded.

Sample size calculation: The sample size was determined using non probability, characteristic, and convenient sampling methods with a 95% confidence level, using the open-epi online statistical tool. A total of 100 patients were analysed for the study, including males and females.

Study Procedure

Information related to patients' age, gender, and IDA-related tests such as Hb, SI, SF, and TIBC were collected from all the subjects. The samples were collected using antecubital venipuncture under aseptic conditions at a temperature of 23-24°C. A 5 mL venous blood sample was drawn from each participant and divided into two tubes: 2 mL was withdrawn into Ethylenediaminetetraacetic Acid (EDTA) tubes for measuring haematological parameters/Complete Blood Count (CBC), while 3 mL was drawn into a plain tubes with no anticoagulant to measure SI, SF, and TIBC. Biochemical tests were performed on samples with low Hb based on WHO guidelines to confirm the diagnosis of IDA [23].

Blood samples were withdrawn from the antecubital vein into glass centrifuge tubes containing oxalate solution (1.34%) as an anticoagulant. After centrifugation at 1500 rpm for 15 minutes, plasma was withdrawn and used for the analysis of serum lipid parameters. Hb, SF, and SI were measured, and TIBC estimation was done by the Ferrozine method, estimating Unsaturated Iron Binding Capacity (UIBC) with the reagent kit available in the market and later calculated using this formula automatically by the instrument: Iron level + UIBC = TIBC (µg/dL) [23]. Lipid estimations (TG, cholesterol, HDL-C, and LDL-C) were done using an enzymatic kit method. LDL values were determined by Fried Wald's formula: $LDL=TC-(HDL+(TG/5))$ [24]. The method of estimation and the cut-off range for all the parameters are given in [Table/Fig-1].

STATISTICAL ANALYSIS

Microsoft Windows 7 was used for data entry. Study findings were explained through tables. The data was statistically analysed with the Statistical Package for the Social Sciences (SPSS), version 26.0. To assess the relationship between the variables, Pearson's correlation was used. To find the significance in categorical data, the Chi-square test and Fischer's-exact test were used. A two-sample

Profiles	Variables	Method of estimation	Cut-off range
IDA profile [23]	Haemoglobin (Hb)	Haematology analyser Mindray 5 part BC-6200 fully automated	<12.0 g/dL
	Serum Iron (SI)	Ferrozine method by fully automated chemistry analyser	<65 µg/L
	TIBC	Calculated by using formula automatically by the instrument. {Iron level+UIBC=TIBC (µg/dL)}	≥450 µg/dL
	Serum Ferritin (SF)	Spectrophotometrically on Beckman coulter chemistry analysers	<10 ng/mL
Lipid profile [24]	Total Cholesterol (TC)	Automated analyser (Fully automatic Erba EM 360 clinical chemistry analyser) by an enzymatic kit method	>200 mg/dL
	Triglyceride (TG)		>150 mg/dL
	HDL-C		<40 mg/dL
	LDL-C	Fried Wald's formula: $LDL=TC-(HDL+(TG/5))$	>130 mg/dL

[Table/Fig-1]: Tabulation of parameters with method and cut-off range.

independent t-test was used to compare the mean values of two different groups. A p-value of <0.05 was considered significant.

RESULTS

The study was carried out on 100 IDA patients to investigate the risk factors related to serum lipid profiles, comprising 38 males and 62 females. Both males and females were found to be aged between 25 to 45 years, with mean ages of 32.92±4.18 and 33.96±5.11 years, respectively. There was no significant difference in parameters such as age, Hb, SI, SF, TIBC, TG, TC, HDL-C, and LDL-C when compared between males and females (p>0.05) [Table/Fig-2].

Variables	Male (n=38)	Female (n=62)	p-value
Age (year)	32.92±4.18	33.96±5.11	0.1914
Hb (mg/dL)	9.16±1.90	8.95±1.73	0.5084
Serum iron (SI) (µg/dL)	45.42±11.35	47.88±9.54	0.2259
TIBC (µg/dL)	512.23±40.69	519.83±39.30	0.3566
Ferritin (ng/mL)	6.30±0.76	6.34±0.92	0.2140
TC (mg/dL)	153.44±65.02	147.51±57.27	0.3741
TG (mg/dL)	121.41±62.48	115.94±56.57	0.4840
HDL-C (mg/dL)	42.37±11.55	41.64±9.35	0.1414
LDL-C (mg/dL)	86.79±41.96	82.56±38.66	0.5615

[Table/Fig-2]: Demographic information, status of anaemia indices, and lipid profile of subject (N=100).

TIBC: Total iron binding capacity; TC: Total cholesterol; TG: Triglyceride; HDL-C: High density lipoprotein; LDL-C: Low density lipoprotein; Two sample t-test (Independent t-test)

In the present study, 82 out of 100 patients, including males and females, showed lower TC and LDL-C levels (<200 mg/dL, and <130 mg/dL, respectively), whereas 18 patients with inclusive of males and females were reported to have higher values of TC, LDL-C, and 20 patients for TG (>200 mg/dL, >130 mg/dL, and >150 mg/dL, respectively). For HDL-C, the majority of patients showed lower levels. However, a substantial number of 38 patients (both male and female) were reported to have higher values of HDL-C (>40 mg/dL, respectively). There were 38 males and 62 females in total. Of the total females, 52 (83.87%) were found to have low levels of TC and LDL, while 30 (78.94%) of the total males exhibited the same. Regarding TG, 52 (83.87%) of total females and 28 (73.68%) of total males showed lower values. A total of 10 females (16.12%) and 8 (21.05%) of males were found to have high levels of TC and LDL. In the case of TG, 10 (16.12%) of females and 10 (26.31%) of total males showed higher values [Table/Fig-3].

Haemoglobin levels showed a significant association with HDL-C and LDL-C with 0.006 and 0.046, respectively, whereas TC and TG failed to exhibit significance with Hb levels (p-value=0.0701). Additionally, SI was found to have a significant association with TC,

Lipid profile		Male (n)	%	Anaemic patients		
				Female	%	Total
TC	<200	30	78.94	52	83.87	82
	>200	8	21.05	10	16.12	18
TG	<150	28	73.68	52	83.87	80
	>150	10	26.31	10	16.12	20
HDL-C	<40	22	57.89	40	64.51	62
	>40	16	42.10	22	35.48	38
LDL-C	<130	30	78.94	52	83.87	82
	>130	8	21.05	10	16.12	18

[Table/Fig-3]: Number of anaemic patients with different lipid parameters.

TC: Total cholesterol; TG: Triglyceride; HDL-C: High density lipoprotein cholesterol; LDL-C: Low density lipoprotein cholesterol

TG, and HDL-C with 0.0130, 0.0130, and 0.0265 ($p < 0.05$), and LDL-C was not found to be significant with SI [Table/Fig-4].

Variables		TC	TG	HDL-C	LDL-C
Hb	p-value	0.0701	0.0701	0.006	0.046
Iron	p-value	0.0130	0.0130	0.0265	0.242
TIBC	p-value	0.594	0.594	0.929	0.598
Ferritin	p-value	0.679	0.545	0.873	0.575

[Table/Fig-4]: Association between lipid profile and IDA patients.

Hb: Haemoglobin; TC: Triglyceride; HDL-C: High density lipoprotein-cholesterol; LDL-C: low density lipoprotein-cholesterol

Pearson's correlation was used to check the association between lipid profile with Hb, iron and TIBC by Fisher's-exact test and lipid profile with ferritin by Pearson's test
p-value is significant at < 0.05

Haemoglobin levels showed a significantly positive correlation with TG (r-values 0.263, p-values 0.008), HDL-C (r-values 0.253, p-values 0.01), and LDL-C (r-values 0.215, p-value 0.03), respectively. The other parameters such as SI and TIBC showed a negative correlation with TG, TC, HDL, and LDL-C ($p > 0.05$) [Table/Fig-5].

Variables		TC	TG	HDL-C	LDL-C
Hb	Correlation covariance	0.193	0.263	0.253	0.215
	p-value	0.053	0.008**	0.01*	0.03*
Iron	Correlation covariance	-0.1024	-0.0949	-0.141	-0.0905
	p-value	0.31	0.34	0.160	0.37
TIBC	Correlation covariance	-0.0128	-0.0004	-0.0143	-0.0154
	p-value	0.898	0.996	0.887	0.878
Ferritin	Correlation covariance	-0.00075	0.0053	0.0014	-0.0023
	p-value	0.994	0.957	0.988	0.981

[Table/Fig-5]: Correlation between the lipid profiles with IDA patients.

Hb: Haemoglobin; TC: Triglyceride; HDL-C: High density lipoprotein cholesterol; LDL-C: Low density lipoprotein cholesterol; Correlation by correlate bivariate method; *p-value is significant at < 0.05 ; **p-value is highly significant < 0.01

DISCUSSION

Iron deficiency is one of the most prevalent public health issues, and it is related to several health issues such as dyslipidemia, especially in developing nations such as India [2]. IDA, being a nutritional disorder, is common in both men and women. In the current paper, 100 patients ranging from 25 to 45 years of age were included, with 38 being males and 62 being females. It is important to note that females are more likely to show IDA compared to males in the population, as this observation is not only seen in the present study but also in several other studies [25-27]. Due to insufficient information regarding this among Indian people, more work is required in this area.

According to current findings, 84% of females and 79% of males were found to have low levels of TC and LDL. In the case of TG, 84% of females and 73% of males showed lower values; hence, the prevalence with respect to gender is nevertheless the same. The results indicate that the maximum number of IDA patients, including males and females, showed lower levels of TC, TG, and

LDL-C. Sandeep N et al., observed lower levels of TC, HDL, LDL, and TG levels in patients with anaemia [28], indicating an inverse relationship between anaemia and lipid profile. Similarly, Shirvani M et al., showed that the amount of TG and TC in elderly subjects with IDA was less compared to other people [29].

A reduction in mean TC, TG, and LDL-C levels was observed in anaemic patients, implying that anaemia leads to the reduction of lipid parameters. The underlying mechanism behind dyslipidemia in IDA patients may be due to several factors, such as a lack of RBCs in the blood. Several theories and postulates have been proposed based on a few reports, as discussed here. According to Ohira Y et al., the concentration of RBCs may affect cholesterol synthesis or mobilisation from tissue to plasma [30].

Ohkawa R et al., obtained similar findings, indicating that red blood cells (RBCs), which carry large quantities of free cholesterol in their membrane, play an important role in reverse cholesterol transport. However, due to the lack of RBCs in anaemic patients, it may indicate a deprivation of cholesterol in the serum. The exact role of RBCs in systemic cholesterol metabolism is poorly understood [31]. Researchers have found that a lower lipid profile correlates with anaemia severity and iron levels. Additionally, a lower lipid score has been linked with anaemia [32].

High levels of TC and LDL were observed in relatively fewer patients, i.e., 16% of females and 21% of males. In the case of TG, 16% of females and 31% of males showed higher values. Thus, it can be inferred that IDA patients had significantly lower levels of TC, TG, and LDL, as very few patients showed higher levels of these. Therefore, regular evaluation and follow-up are required for lipid estimation in IDA patients. A significant association was observed between haemoglobin (Hb) levels, high-density lipoprotein cholesterol (HDL-C), and LDL-C, whereas TC and TG failed to exhibit the same. However, Hb showed a negative correlation with TC and a positive correlation with TG, HDL-C, and LDL-C.

A previous study found a significant association of Hb with the lipid profile, which included TC, LDL, HDL, and TGs ($p < 0.05$) [33]. A researcher found statistically significant TG, TC, and LDL when compared with IDA, and these values were strongly correlated with Hb levels [34]. It is interesting to note that Choi JW et al., revealed the positive correlation of Hb levels with serum TC in the IDA group [35].

The study also indicates that Serum Iron (SI) showed a significant association with TC, TG, and HDL-C ($p < 0.05$); however, LDL-C was not found to be significant with SI ($p > 0.05$). The iron levels did not meet the correlation criteria with any of these parameters. Some papers report that SI levels, when checked in female students, were found to be negatively correlated with TG but positively correlated with HDL-C, LDL-C, and TC [36]. However, Suliburska J. et al., found no significant correlation between serum and dietary concentrations of iron and serum cholesterol or TG levels [37]. Meanwhile, Stangle and Kirchgessner reported that hypertriglyceridemia in mice is associated with low-iron diets [38].

Another study conducted on paediatric patients showed that poor iron status appeared to correlate with a poorer lipid response compared to non iron deficient patients [39]. Conversely, Ece A. et al., found that iron deficiency has no effect on the lipid profile. They suggested that a low-iron diet causes a loss of energy and protein, leading to a calorie-deficient diet, which results in the low atherogenic serum lipid profile of IDA patients. This is not a direct result of iron deficiency itself but is related to decreased energy and protein intakes [22]. In the study conducted by Mitrache C. et al., IDA patients showed a significantly lower level of serum cholesterol [40].

Several theories have been postulated to explain the link between dyslipidemia and IDA. Possibly, the impaired carnitine mechanism due to IDA also becomes the culprit for dyslipidemia. Lower serum cholesterol may be related to decreased hepatic synthesis. The exact mechanism by which iron regulates or functions in lipid metabolism

has not yet been established [41]. Another review describes cross-talk between iron and lipid pathways, including alterations in cholesterol, sphingolipid, and lipid droplet metabolism in response to changes in iron levels [14]. A separate study conducted on mice related to hepatic iron loading and cholesterol biosynthesis suggests that iron loading increases liver cholesterol synthesis by upregulating seven enzymes. They found that cholesterol is positively correlated with hepatic iron and therefore with serum cholesterol [42]. Some researchers believe that iron plays a role in hepatic lipogenesis [8]. According to Kim SH et al., high consumption of iron intensified hyperlipidemia and induced fatty liver changes [43].

Limitation(s)

The main constraints of the present study include the limited sample size. Additionally, no comparisons between the patient groups and control groups were observed. A separate study related to oral iron supplements and their impact on changes in the lipid profile is greatly needed.

CONCLUSION(S)

The present study concludes that there is a definite correlation between anaemia and lipid abnormalities. The values of the lipid profile parameters, including TG, LDL-C, HDL-C, and TC, in IDA were found to be relatively lower, indicating dyslipidemia. Based on this result, it is assumed that the decreases in serum lipid concentrations are related to IDA. Understanding the mechanism of TC and TG synthesis with respect to iron levels is an important aspect of IDA and should be taken into consideration for further understanding of the condition. Due to the lack of sufficient research on the interrelationship of lipid metabolism with iron, future studies are warranted to find out the exact mechanism behind it.

Acknowledgement

The authors would like to express their sincere gratitude to the patients, who formed the most integral part of the work and were kind and cooperative. Also, to the administrative staff of Parul Sevashram Hospital who supported the aforementioned study.

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PLAGIARISM CHECKING METHODS: [\[Jain H et al.\]](#)

- Plagiarism X-checker: Jul 06, 2023
- Manual Googling: Dec 18, 2023
- iThenticate Software: Jan 02, 2024 (16%)

ETYMOLOGY: Author Origin**EMENDATIONS:** 8**AUTHOR DECLARATION:**

- Financial or Other Competing Interests: Funded by CR4D (Centre of Research for Development), Parul University, Vadodara, Gujarat, India
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Jul 03, 2023**Date of Peer Review: **Sep 30, 2023**Date of Acceptance: **Jan 05, 2024**Date of Publishing: **Mar 01, 2024**