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Original Article - Role of Serum Iron and Serum Ferritin in Type II Diabetes

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Abstract
Aims and objectives- to study the role of serum iron and ferritin in diabetics and its correlation with duration glycemic status, complications and medication status**.**
Material and methods- this study was carried out in the Department of Medicine, G.R. Medical College and J.A. Group of Hospitals, Gwalior with ethical approval. This study included 50 diagnosed patients of diabetes mellitus belonging to various age groups and both sexes, and 25 age and sex matched healthy control cases were taken. All cases and control were subjected to detailed clinical history and, Clinical history and examination regarding duration of diabetes and treatment taken and history of other diseases and diabetic complication. inclusuion, exclusion and diagnostic criteria was followed and all patients were planned for routine and special laboratory investigations and then data was studied and results are as follows.
Results- The mean age for the patients was 55.30yrs, whereas the mean age for the control group was 53.28yrs.Among diabetic patients, out of 50 individuals 32 were male and 18 were female. In control group, there were 16 male and 9 female individuals. The mean serum ferritin level in diabetic group was 170.242ng/ml and in control group was 43.28ng/ml. The mean serum iron of patients (group 1) was 110.884mcg/dl and that of group 2 was 75.8 mcg/dl. The difference was statistically highly significant for both serum ferritin and serum iron ($P < 0.001$ and $P=0.014$ respectively). A statistically significant difference was found in the serum ferritin and serum iron levels ($P=0.044$ and $P=0.008$ respectively) between the male and female patients ($P=0.044$ and $P=0.008$ respectively).The correlation of serum ferritin and serum iron levels with HbA1C was done. Both serum ferritin and serum iron levels did not show any correlation with HbA1C ($P=0.985$ and $P=0.463$ respectively).No significant correlation was found between serum ferritin and serum iron levels and duration of diabetes. Significant correlation was found between serum ferritin and serum iron levels and increasing number of complications of diabetes ($P=0.002$ and $P=0.005$ respectively).
Patients not taking any regular treatment had significantly higher levels of serum ferritin and serum iron than those taking regular treatment. But no significant difference was seen in serum ferritin and serum iron levels among those taking OHA and those on insulin.
Conclusion-Increased levels of serum ferritin and serum iron were observed in diabetic patients in our study adding credibility to the hypothesis that iron metabolism might participate in the etiology of insulin resistance. Levels of serum ferritin and serum iron were not correlating with the glycemic control of diabetic patients (HbA1c) and according to our study hence could not be used for the same.

Keywords: Diabetes Mellitus type 2, Serum Iron, Serum Ferritin, HBA1c

1. Introduction
Diabetes Mellitus is assuming epidemic proportions worldwide. Approximately one-fifth of the world diabetics are in India (WHO) and the incidence of the disease is increasing day by day. Diabetes is responsible for increased cardiovascular mortality and over all low quality of life. 90% of diabetes mellitus cases are of type 2. Insulin resistance is the main pathogenic mechanism for Type 2 Diabetes Mellitus. The underlying causes for development of insulin resistance are not well defined, but both the genetic and environmental factors play a role. Clinical and epidemiological data implicate inflammatory mediators are also associated with insulin resistant state [1, 2]. Evidence also suggests a link between body iron excess and insulin metabolism[3]. Ferritin, the major iron storage protein, plays a key role in iron metabolism [4, 5]. Serum ferritin concentration provides an indirect estimate of body iron stores because it is highly correlated with bone marrow iron [6, 7]. Ferritin is also a positive acute–phase reactant and increases in various acute or chronic disease conditions [4, 5]. Elevated serum ferritin levels have been found in much chronic inflammation - related diseases [8, 9]. Studies have shown an association between serum ferritin and one or more of the metabolic syndrome features [10]. Elevated serum ferritin levels independently predicted the incidence of Type 2 Diabetes

Mellitus in a prospective study in apparently healthy men and women [11, 12]. Elevated ferritin level has also been associated with hypertension, dyslipidemia [13], elevated fasting insulin[14], glucose[3] and adiposity [13]. Serum Ferritin has even been suggested to be considered as a part of insulin resistance syndrome. Also, elevated serum ferritin concentrations early in gestation are associated with increased risk of gestational diabetes mellitus [15].Diet and lifestyle play a major role in Type 2 Diabetes Mellitus. Indian population has high prevalence of iron deficiency anaemia. Also Indian dietary habits are different from the west, mainly comprising of vegetarian food and less of meat products. However, the incidence of diabetes is increasing in India. We examined the association of serum iron and serum ferritin to insulin resistance and diabetes mellitus in Indian patients.

1.2. Aims and Objectives

1. Evaluation of serum iron and serum ferritin levels in diagnosed cases of Type 2 diabetes mellitus. 2. To compare serum iron and serum ferritin levels in patients of Type2 diabetes with healthy population.3. To correlate serum iron and serum ferritin levels with glycemic control.4. To correlate serum iron and serum ferritin levels with duration of Type2 diabetes.5. To correlate the complications of Type2 diabetes with serum iron and serum ferritin levels.6. To correlate the serum iron and serum ferritin levels in Type2 diabetics taking oral hypoglycemic agents and/or insulin.

2. Material and Method- The present study was carried out in the Department of Medicine, G.R. Medical College and J.A. Group of Hospitals, Gwalior. It included indoor patients as well as OPD patients. This study included 50 diagnosed patients of diabetes mellitus belonging to various age groups and both sexes, and 25 age and sex matched healthy control cases were taken. All cases and control were subjected to detailed clinical history and examination as follows: Age and sex, Family History/O smoking, alcohol or drug intake., Height, weight, BMI, BP in supine and standing position, Clinical history and examination regarding duration of diabetes and treatment taken and history of other diseases and diabetic complication like retinopathy, coronary artery disease, peripheral vascular disease, cerebrovascular disease etc. as and when required.

2.2. Selection of Subjects

2.3. Inclusion Criteria-Patients of age > 13 years**,** Type 2 Diabetes Mellitus presenting with or without complications (except patients with diabetic nephropathy)

2.4. Exclusion Criteria-Patients of age <13 yrs, Anaemic patients, Pregnancy, Chronic Inflammatory state or any other systemic illness like rheumatoid arthritis, malignancy, Chronic smokers, Chronic alcoholics, Chronic liver Disease including those suffering from hepatitis B or C., Chronic Renal Disease, Patient on iron therapy

2.5. Diagnostic Criteria

Anaemia was defined as hemoglobin levels < 13 gm % for men and < 12 gm % for women (WHO Definition) [145].

2.6. Criteria for diagnosis of Diabetes Mellitus [146]

- Symptoms of diabetes plus random blood glucose concentration >= 11.1 mmol/L (200mg/dL)[a] *or*
- Fasting plasma glucose >= 7.0 mmol/L (126 mg/dL)[b] *or*
- Two – hour plasma glucose >=11.1 mmol/L (200 mg/dL) during an oral glucose tolerance test. [c]

[A] Random is defined as without regard to time since the last meal.

[B] Fasting is defined as no calorie intake for at least 8 hrs.

[C] The test should be performed using a glucose load containing the equivalent of 75 gm anhydrous glucose dissolved in water, not recommended for routine clinical use.

2.7. Laboratory Investigations

Baseline Investigation**:** Blood sugar – fasting & post – prandial, Glycosylated hemoglobin (HbA1c),Urine(routine / microscopy, micro albuminuria)**,** Kidney function test – viz. blood urea, serum creatinine, Liver function test (Serum Bilirubin, SGPT),Serum Lipid profile – after 12 hours of overnight fasting, C - Reactive protein (CRP),Chest X – ray, Ultrasound Abdomen and ECG (where necessary).

2.8. Hematological Investigation Hemoglobin, Complete blood count and differential count, Erythrocyte sedimentation rate, Erythrocyte Indices: (Serum iron, Serum ferritin)**.**C-Reactive Protein (CRP) was qualitatively measured by slide latex agglutination method. Cut off was taken as 6mg /liter. We excluded patients with a baseline CRP > 6mg/dl, because inflammation may increase serum ferritin and serum transferrin, and also those with a "very low" ferritin, which might be due to anemia (<15 mcg/l), and a "very high" ferritin which might be due to hemochromatosis (>500mcg/l). Blood Pressure was taken in a supine position after 5 min of rest and after 3 minutes of standing. BP was also measured in the lower limb to find the Ankle Brachial Index. Weight, and height were measured in lightly clad participants, and BMI was calculated. Glycaemic control was assessed by glycated hemoglobin (HbA1C). Chronic liver disease was excluded by taking history and clinical examination and liver function test if required. Chronic renal disease was excluded by taking history and measuring blood urea and serum creatinine. Serum Ferritin was measured in the laboratory by chemiluminescence method (N = 15-300 ng/ml). Serum iron (N = 50-150 mcg/dl) was measured in laboratory by chemiluminescence method. We obtained the serum ferritin and serum iron levels of the patients and compared it with the healthy population. Serum ferritin and iron levels were also compared between the male and female patients to see for any differences between the two groups in diabetic patients. We divided the diabetic population into 3 groups, according to the control of their sugar levels, into HbA1C <7, HbA1C 7 to 9, and HbA1C >9, and then compared the serum ferritin and iron levels within the three groups. We also divided the diabetic patients into 3 groups, according to the duration of diabetes, into duration < 5yrs, 5 to 10yrs and >10yrs, and then compared the serum ferritin and iron levels within the three groups. Serum ferritin and iron levels were also compared between the diabetic patients presenting with various complications of diabetes. The serum feritin and iron levels were also compared between the diabetic patients not taking any treatment and those taking regular treatment. The results obtained were analyzed by applying statistical methods like Chi square test, student's t test, N Par test, Mann Whitney test. Whatsoever was appropriate using SPSS software.

Observations The present study "To study the role of serum iron and serum ferritin in Type II Diabetes" was conducted at the Medicine Department of GRMC and JAH, Gwalior from September 2007 to September 2008. A total of 75 individuals were recruited in this study; 50 belonging to cases group (Group1) satisfying all inclusion and exclusion criteria as mentioned above, and the rest 25 healthy individuals

belonging to the control group (Group 2). For analytical purpose, the serum ferritin levels of the population under study were divided into 3 groups- serum ferritin <150ng/ml (normal), serum ferritin 150 to 300ng/ml (high normal) and serum ferritin >300ng/ml (abnormal). The serum iron levels were also divided into 3 groups- serum iron 50 to 100mcg/dl(normal), serum iron 100 to 150mcg/dl(high normal) and serum iron >150mcg/dl (abnormal).

Table 1: Demographic Profile of Cases (Group 1)

Age Group(Yrs)	Male	Female	Total
<40	1	0	1
41-50	12	7	19
51-60	14	4	18
61-70	4	6	10
71-80	1	1	2

Table 2: Demographic Profile of Controls (Group 2)

Age Group (Yrs)	Male	Female	Total
<40	1	1	2
41-50	6	3	9
51-60	6	2	8
61-70	2	3	5
71-80	1	0	1

Table 3: Sex Distribution of the Population

Sex	Group		Total
	Cases	Control	
Male	32	16	48
Female	18	9	27
Total	50	25	75

Table 4: Distribution of Serum Ferritin Levels in the Population

Serum Ferritin (ng/ml)	Cases (Group 1)			Controls (Group 2)		
	Males	Females	Total	Males	Females	Total
	n=32	n=18	n=50	n=16	n=9	n=25
<150	14(43.8%)	12(66.7%)	26(52%)	16(100%)	9(100%)	25(100%)
150 – 300	9(28.1%)	4(22.2%)	13(26%)	0	0	0
>300	9(28.1%)	2(11.1%)	11(22%)	0	0	0

Table 5: Mean Serum Ferritin Levels (Ng/Ml) In Cases and Controls

	Mean	S.D.	S.E.	95% Ci		Min.	Max.
				Lower Bound	Upper Bound		
Cases	170.242	119.549	16.907	136.267	204.217	16	458
Controls	43.28	22.819	4.564	33.861	52.699	17	125

The mean serum ferritin level in diabetic patients was 170.242ng/ml.
The mean serum ferritin level in controls was 43.28ng/ml.

Table 6: Distribution of Serum Iron Levels in the Population

Serum Iron (mcg/dl)	Cases (Group 1)			Controls (Group 2)		
	Males	Females	Total	Males	Females	Total
	n=32	n=18	n=50	n=16	n=9	n=25
50 – 100	13(40.6%)	12(66.7%)	25(50%)	13(61.25%)	6(66.7%)	19(76%)
100 – 150	10(31.3%)	6(33.3%)	16(32%)	3(18.75%)	3(33.3%)	6(24%)
>150	9(28.1%)	0	9(18%)	0	0	0

Table 7: Mean Serum Iron Levels (Mcg/Dl) In Cases and C0ntrols

	Mean	S.D.	S.E.	95% Ci		Min.	MAX.
				Lower Bound	Upper Bound		
Cases	110.884	58.267	8.240	94.325	127.443	33	246
Controls	75.8	30.488	6.097	63.215	88.385	33	125

The mean serum iron level in diabetic patients was 110.884mcg/dl. The mean serum iron level in controls was 75.8mcg/dl.

Table 8: Comparison of Serum Ferritin and Iron Levels between Cases and Control

	Cases	Control	P- Value	Significance
Serum Ferritin	170.242	43.28	< 0.001	Highly Significant
Serum Iron	110.884	75.8	0.014	Significant

The serum ferritin in the group 1 was much more than the control group and the difference was statistically highly significant (P<0.001). Also there was statistically significant difference (P=0.014) in the levels of serum iron of the patients group and control group with levels higher in the former as compared to the latter.

Table 9: Comparison of Serum Ferritin and Iron Levels between Males and Females in Diabetic Patients

	Male	Female	P- Value	Significance
Serum Ferritin	194.11	127.82	0.044	Significant
Serum Iron	127.75	80.89	0.008	Significant

Serum ferritin and serum iron levels were higher in males as compared to females. The difference was statistically significant (P=0.044 and P=0.008 for serum ferritin and serum iron respectively).

Table 10: Distribution of Diabetic Patients According To Their Glycaemic Control

Glycaemic Control	Hba1c	No. Of Patients
Good Control	<7	11
Moderate Control	7 to 9	21
Poor Control	>9	18

Table 11: Serum Ferritin Levels in Diabetic Patients According To Their Glycaemic Control

Serum Ferritin (ng/ml)	Hba1c<7		Hba1c 7TO9		Hba1c>9	
	n=11	%	n=21	%	n=18	%
<150	7	63.6	10	47.6	9	50
150 – 300	2	18.2	8	38.1	3	16.7
>300	2	18.2	3	14.3	6	33.3

Increase in serum ferritin levels is observed with progressively poor glycemic control. However, the difference observed is not significant (P=0.391).

Table 12: Serum Iron Levels in Diabetic Patients According To Their Glycaemic Control

Serum Iron (mcg/dl)	Hba1c<7		Hba1c 7TO9		Hba1c>9	
	n=11	%	n=21	%	n=18	%
50 – 100	6	54.5	12	57.1	7	38.9
100 – 150	2	18.2	7	33.3	7	38.9
>150	3	27.3	2	9.6	4	32.2

Increase in serum ferritin levels is observed with progressively poor glycemic control. However, the difference observed is not significant (P=0.528).

Table 13: Correlation of Serum Ferritin and Iron with Hba1c in Diabetic Patients

	Correlation Coef.	P-Value	Significance
Serum Ferritin	0.003	0.985	Not Significant
Serum Iron	0.106	0.463	Not Significant

Both serum ferritin and serum iron levels did not show any correlation with HbA1C, the correlation coefficient being r = +0.003 and P-value of 0.985 and r = +0.106 and P = 0.463 respectively.

Table 14: Distribution of Diabetic Patients According To Their Duration of Diabetes

Duration Of Diabetes	No Of Patients
<5 YRS	25
5 to 10 YRS	19
>10 YRS	6

Table 15: Serum Ferritin Levels in Diabetic Patients According To Their Duration of Diabetes

Serum Ferritin (ng/ml)	<5yrs		5 To 10 Yrs		>10yrs	
	n=25	%	n=19	%	n=6	%
<150	14	56	9	47.36	3	50
150 – 300	7	28	5	26.32	1	16.7
>300	4	16	5	26.32	2	33.3

Increase in serum ferritin levels is observed with increasing duration of diabetes. However, the difference observed is not significant (P=0.858).

Table 16: Serum Iron Levels in Diabetic Patients According To Their Duration of Diabetes

Serum Iron (Mcg/Dl)	<5yrs		5 To 10 Yrs		>10yrs	
	n=25	%	n=19	%	n=6	%
50 – 100	15	60	7	36.84	3	50
100 – 150	8	32	7	36.84	1	16.7
>150	2	8	5	26.32	2	33.3

Increase in serum iron levels is observed with increasing duration of diabetes. However, the difference observed is **not significant** (P=0.326).

Table 17: Table Showing Various Complications Seen In Diabetic Patients

Complication	No Of Patients
Retinopathy	21
Neuropathy	19
CAD	26
CVA	3

Table 18: Serum Ferritin and Serum Iron Levels in Various Complications in Diabetic Patients

Complication	Serum Ferritin(ng/ml)			Serum Iron(mcg/dl)		
	<150	150-300	>300	50-100	100-150	>150
	No (%)	No (%)	No (%)	No (%)	No (%)	No (%)
Retinopathy n=21	5(23.81)	6(28.57)	10(47.62)	7(33.33)	8(38.1)	6(28.57)
Neuropathy n=19	4(21.05)	6(31.58)	9(47.37)	7(36.85)	4(21.05)	8(42.10)
Cad n=26	8(30.77)	8(30.77)	10(38.46)	7(26.93)	11(42.3)	8(30.77)
Cva n=3	0	2(66.67)	1(33.33)	1(33.33)	0	2(66.67)

The difference observed is not significant (P=0.830 for serum ferritin and P=0.607 for serum iron).

Table 19: Distribution of Diabetic Patients According To Number of Complications

5No Complication	13
1 Complication	19
2 Complication	7
>=3 Complication	11

Table 20: Serum Ferritin and Serum Iron Levels in Diabetic Patients According To Increasing No Of Complications

Number Of Complication	Serum Ferritin(ng/ml)			Serum Iron(mcg/dl)		
	<150	150-300	>300	50-100	100-150	>150
	No (%)	No (%)	No (%)	No (%)	No (%)	No (%)
1 COMPLIC. n=19	11(57.89)	7(36.85)	1(5.26)	11(57.89)	8(42.10)	0
2 COMPLIC. n=7	3(42.86)	2(28.57)	2(28.57)	4(57.14)	1(14.280	2(28.57)
>=3 COMPLIC. n=11	0	3(27.28)	8(72.72)	1(9.09)	5(45.46)	6(54.55)

Elevated serum ferritin and serum iron levels are seen with increasing number of complications. The difference observed is significant for both serum ferritin and serum iron (P=0.002 and P=0.005 respectively).

Table 21: Table Showing Treatment Profile of Diabetic Patients

No Treatment	11
Irregular Treatment	6
Regular Treatment	33

Table 22: Serum Ferritin and Serum Iron Levels in Diabetic Patients According To Their Treatment Profile

	Serum Ferritin(ng/ml)			Serum Iron(mcg/dl)		
	<150	**150-300**	**>300**	**50-100**	**100-150**	**>150**
	No (%)	**No (%)**	**No (%)**	**No (%)**	**No (%)**	**No (%)**
No Treatment n=11	2(18.18)	3(27.28)	6(54.54)	1(9.09)	6(54.55)	4(36.36)
Irregular Treatment n=6	0	4(66.67)	2(33.33)	1(16.67)	4(66.66)	1(16.67)
Regular Treatment n=33	24(72.73)	6(18.18)	3(9.09)	23(69.69)	6(18.18)	4(12.13)

Decrease in serum ferritin and serum iron levels is seen with use of regular treatment. The difference observed is significant for both serum ferritin and serum iron (P<0.001 and P=0.003 respectively).

Table 23: Table Showing Diabetic Patients Taking Oha or Insulin Regularly

On Regular Treatment (N=33)	
Taking Oha Regularly	29
Taking Insulin Regularly	4

Table 24: Serum Ferritin and Serum Iron Levels in Diabetic Patients Taking Regular Treatment on Oral Hypoglycemic Agents or Insulin Therapy

	Serum Ferritin(ng/ml)			Serum Iron(mcg/dl)		
	<150	**150-300**	**>300**	**50-100**	**100-150**	**>150**
	No (%)	**No (%)**	**No (%)**	**No (%)**	**No (%)**	**No (%)**
Taking Oha Regularly n=29	23(79.32)	49(13.79)	2(6.89)	21(72.21)	6(20.70)	2(6.89)
Taking Insulin Regularly N=4	1(25)	2(50)	1(25)	2(50)	0	2(50)

The difference observed is not significant (P=0.073 for serum ferritin and P=0.069 for serum iron).

3. Discussion-Diabetes Mellitus is one of the most common endocrinal disease with an increasing incidence all over the world. The role of iron in Type2 Diabetes Mellitus is debated from one of the factors for pathogenic mechanism [12, 18, 19, 20] to a nonspecific inflammatory marker [1, 2, 4, 5, 8, 9] of metabolic syndrome. Abnormalities in iron status have been described in Diabetes Mellitus and generated renewed interest. Increased serum ferritin levels have been found in Type2 Diabetes Mellitus in many studies. This study was undertaken to determine the serum ferritin and serum iron levels in Type2 Diabetes Mellitus. Body iron status varies with geographical area and dietary habits. To study the role of serum iron and serum ferritin in adult Type2 Diabetes Mellitus cases, this study was conducted at the Medicine Department of GRMC and JAH, Gwalior. We studied 50 patients of Type2 Diabetes Mellitus for serum iron and serum ferritin levels in addition to other laboratory and clinical parameters. 25 healthy controls which were age and sex matched were taken. The mean age for the patients was 55.30yrs±8.812, whereas the mean age for the control group was 53.28yrs±10.06.Among diabetic patients, out of 50 individuals 32 were male and 18 were female. In control group, there were 16 male and 9 female individuals. For analytical purpose, the serum ferritin levels of the population were divided into 3 groups: normal (serum ferritin <150ng/ml), high normal (150-300ng/ml) and abnormal (>300ng/ml). The serum iron levels of the population were also divided into 3 groups: normal (serum iron 50-100mcg/dl), high normal (100-150mcg/dl) and abnormal (>150mcg/dl).

3.1. Comparison of Serum Ferritin and Serum Iron Levels between Cases and Control out of the 50 diabetic patients, 26(52%) had normal serum ferritin, 13(26%) had high normal serum ferritin, and 11(22%) had abnormal serum ferritin levels. All 25 controls (100%) had normal serum ferritin levels. The serum iron levels were normal in 25 patients (50%), high normal in 16 (32%) and abnormal in 9(18%) patients. Among controls 19(76%) had normal and 6(24%) had high normal serum iron levels. The mean (S.D) serum ferritin level in diabetic group was 170.242ng/ml (± 119.549). The mean (S.D) serum iron of patients (group 1) was 110.884mcg/dl (± 58.267). The mean (S.D) serum ferritin level in control group was 43.28ng/ml (± 22.819). The mean (S.D) serum iron of group 2 was 75.8 mcg/dl (± 30.488). The serum ferritin in the diabetic group was higher than the control group and the difference was statistically highly significant (P<0.001). Also there was statistically significant difference (P=0.014) in the levels of serum iron of the patients group and control group with levels higher in the former as compared to the latter. The results of our study were in agreement with the studies conducted earlier. Studies done by Fernandez Real *et al*. [18], Bozzini C *et al*. [19], Jiang R *et al.* [20]. observed an important and a highly significant association between serum ferritin and Diabetes Mellitus. Also Fumeron F *et al*. [12] found that serum ferritin is significantly associated with insulin resistance syndrome thus having high risk of development of Diabetes Mellitus and metabolic syndrome. Sharifi *et al* [22]. Also found an elevation in serum ferritin in prediabetes stage, before the occurance of overt diabetes mellitus. Serum ferritin and iron levels were also compared between the male and female patients (but not the whole population), to see for any differences the two groups in diabetic patients. The mean serum ferritin level in the males was 194.11ng/ml whereas the mean serum ferritin found in the females was 127.82ng/ml. The difference was found to be statistically significant (P=0.044). The mean serum iron level in the males was 127.75mcg/dl whereas the mean serum iron

found in the females was 80.89mcg/dl. The difference was found to be statistically significant (P=0.008).The difference in the iron profile in relation to the sex of the patients has also been observed by Zumin Shi *et al*. [11]. Their study has found raised iron stores (serum ferritin) and iron intake to be associated with increased risk of developing Diabetes Mellitus in Chinese women but not in men. Also Chen X *et al*. [15] had observed high serum ferritin levels in early pregnancy to be associated with increased risk of developing Gestational Diabetes Mellitus. In the study done by Sharifi F *et al*. [22], there was not statistically significant difference in serum ferritin in males and females. The difference observed in our study could be because of small sample size. Also we did not take the dietary habits into account. Also although matched for age and BMI, matching regarding family history of diabetes and hypertension was not done. Hence, further studies with bigger sample size and matching for diet with detailed dietary history about the quality and quantity of diet and family history is required to confirm the same findings.

3.2. Serum Ferritin and Serum Iron Levels and Glycemic Control of Diabetic Patients

Diabetic patients were divided into three groups based on their HbA1c: good control was defined as HbA1c of <7% (11 cases, 22%), moderate control as HbA1c of 7-9% (21 cases, 42%), and poor control as HbA1c levels of >9% (18 cases, 36%). The serum ferritin and serum iron levels were then compared within these 3 groups. The difference in the findings was found to be statistically non-significant for serum ferritin (P=0.391) as well as for serum iron (P=0.528). The correlation of serum ferritin and serum iron levels with HbA1C was done. Both serum ferritin and serum iron levels did not show any correlation with HbA1C, the correlation coefficient being r = +0.003 and P-value of 0.985 and r = +0.106 and P = 0.463 respectively. Our results were in harmony with the study done by Sharifi F *et al*. [134] in June 2001 that serum ferritin levels are high in patients of Diabetes Mellitus but not related to the levels of HbA1c and blood glucose control. The mean serum ferritin in diabetics was significantly higher than normal group (101.5±73 vs. 43.5±41.8µg/ml, P<0.001). Diabetic patients were divided into three groups based on their HbA1c and no significant differences were found between these three groups regarding ferritin levels. But our results were in conflict with the two studies done both by Fernandez Real *et al*. [3, 21]. In the first, Fernandez [3] in 1998 studied the relationship between serum ferritin and the results of glucose tolerance test and insulin sensitivity in healthy subjects. Such results have also been reported by Kim *et al*. [16]. They suggest that serum ferritin may also be an independent determinant of poor metabolic control in diabetic patients. This difference with our study can be due to our exclusion criteria such as anemia and severe diabetic complications that had not been considered in other studies. In the second[21], Fernandez *et al*. studied the effect of ferritin reduction by bloodletting on insulin sensitivity and HbA1c levels in diabetic patients. In this study the positive effect of ferritin reduction on blood glucose control was used for confirmation of the probable role of ferritin in DM pathogenesis but, the use of bloodletting may affect total hemoglobin level and HbA1c as well, so the use of HbA1c as a marker of blood glucose control has not been appropriate.

3.3. Serum Ferritin and Serum Iron Levels and Duration of Diabetes in Diabetic Patients

We divided the diabetic patients into three groups according to their duration of diabetes into duration <5yrs (25 cases, 50%), duration 5 to 10yrs (19 cases, 38%) and duration >10yrs (6 cases, 12%). The serum ferritin and serum iron levels were then compared within these 3 groups. The difference in the findings was found to be statistically non-significant for serum ferritin (P=0.858) as well as for serum iron (P=0.326).

3.4. Serum Ferritin and Serum Iron Levels and Complications in Diabetic Patients The serum ferritin and serum iron levels were compared among the diabetic patients presenting with various complications considered in our study (retinopathy, neuropathy, CAD, CVA). Diabetic nephropathy was not taken into account as it was a part of the exclusion criteria. 21 patients had retinopathy. Out of these the serum ferritin levels were normal in 5(23.81%), high normal in 6(28.57%) and abnormal in 10(47.62%). The serum iron levels were normal in 7(33.33%), high normal in 8(38.1%) and abnormal in 6(28.57%). 19 patients had neuropathy, out of which the serum ferritin levels were normal in 4(21.05%), high normal in 6(31.58%) and abnormal in 9(47.37%). The serum iron levels were normal in 7(36.85%), high normal in 4(21.05%) and abnormal in 8(42.10%). 26 patients had CAD, out of which the serum ferritin levels were normal in 8(30.77%), high normal in 8(30.77%) and abnormal in 10(38.46%). The serum iron levels were normal in 7(26.93%), high normal in 11(42.3%) and abnormal in 8(30.77%). 3 patients had CVA, out of which the serum ferritin levels were normal in none, high normal in 2(66.67%) and abnormal in 1(33.33%). The serum iron levels were normal in 1(33.33%), high normal in none and abnormal in 2(66.67%).The difference found in the serum ferritin and serum iron levels in various complications was statistically not significant with P values of 0.830 and 0.607 respectively. Serum ferritin and serum iron levels were also compared among diabetic patients according to increasing number of complications. Out of the 19 patients having any 1 of the complications considered in our study, 11(57.89%) had normal serum ferritin levels, 7(36.85%) had high normal and 1(5.26%) had abnormal serum ferritin levels. The serum iron levels were normal in 11(57.89%) and high normal in 8(42.10%). Out of the 7 patients having any 2 complications, 3(42.86%) had normal serum ferritin levels, 2(28.57%) had high normal and 2(28.57%) had abnormal serum ferritin levels. The serum iron levels were normal in 4(57.14%), high normal in 1(14.28%) and abnormal in 2(28.57%) patients. Out of the 11 patients having any 3 or more complications, none had normal serum ferritin levels, 3(27.28%) had high normal and 8(72.72%) had abnormal serum ferritin levels. The serum iron levels were normal in 1(9.09%), high normal in 5(45.46%) and abnormal in 6(54.55%) patients. The difference found was statistically significant for both serum ferritin and serum iron levels with P values of 0.002 and 0.005 respectively. Studies done by Kaye TB et al. [17] also found an elevated serum ferritin concentration to be associated with CAD. A correlation between serum ferritin level and diabetic retinopathy was observed by Canturk Z *et al*. [24].

3.5. Comparing Serum Ferritin and Serum Iron Levels with Treatment in Diabetic Patients

The diabetic patients were divided into three groups according to whether they were not taking any treatment, taking treatment regularly or irregularly. Out of the 11 patients not taking any treatment, 2(18.18%) had normal serum ferritin levels, 3(27.28%) had high normal and 6(54.54%) had abnormal serum ferritin levels. The serum iron levels were

normal in 1(9.09%), high normal in 6(54.55%) and abnormal in 4(36.36%) patients. Out of the 6 patients on irregular treatment, none had normal serum ferritin levels, 4(66.67%) had high normal and 2(33.33%) had abnormal serum ferritin levels. The serum iron levels were normal in 1(16.67%), high normal in 4(66.66%) and abnormal in 1(16.67%) patient. Out of the 33 patients taking regular daily treatment, 24(72.73%) had normal serum ferritin levels, 6(18.18%) had high normal and 3(9.09%) had abnormal serum ferritin levels. The serum iron levels were normal in 23(69.69%), high normal in 6(18.18%) and abnormal in 4(12.13%) patients. Statistically significant difference was found in the serum ferritin and serum iron levels within these groups with P value of <0.05 for both serum ferritin and serum iron. Out of the 33 patients on regular treatment, 29 were taking OHA and 4 were taking insulin. Among the patients taking OHA, 23(79.32%) had normal serum ferritin levels, 4(13.79%) had high normal and 2(6.89%) had abnormal serum ferritin levels. The serum iron levels were normal in 21(72.21%), high normal in 6(20.7%) and abnormal in 2(6.89%) patients. Out of the 4 patients taking insulin, 1(25%) had normal serum ferritin levels, 2(50%) had high normal and 1(25%) had abnormal serum ferritin levels. The serum iron levels were normal in 2(50%), high normal in none and abnormal in 2(50%) patients. The difference found in the serum ferritin and serum iron levels was statistically not significant with P values of 0.073 and 0.069 respectively. As ferritin levels can increase in chronic inflammatory states, we excluded patients with coexisting chronic illnesses. We also excluded patients with CRP > 6mg/dl again to rule out inflammatory state.

Increased levels of serum ferritin and serum iron were observed in diabetic patients in our study and add credibility to the hypothesis that iron metabolism might participate in the etiology of insulin resistance. An insulin resistance phenotype has been associated with liver iron overload, and it is characterized by normal transferrin saturation, normal transferrin, and high ferritin in patients without genetic hemochromatosis. Our results suggest that serum ferritin and iron could be added to routine evaluation of Diabetes Mellitus and those having prediabetes; this would help us identify a subgroup of individuals at risk of iron related tissue damage, in whom further investigations may be appropriate. As a result, iron related damage may be prevented by inexpensive therapeutic approach such as phlebotomy. Also since iron metabolism might have a role in the pathogenesis of diabetes, the indiscriminate use of iron preparations should be avoided and reduced dietary intake of iron especially in men with additional risk factors for type2 diabetes is advisable. But still, more comprehensive and on a larger population of patients, studies are needed to confirm the above. The limitations in our study were small sample size, history of blood donation and dietary history was not taken which could have been an important reason for low or high serum ferritin in that particular patient, patients were not matched for their area of residence (socio-economic) status which can have an effect on serum ferritin (iron stores) levels despite of them having hemoglobin in non anaemic range, ours was a cross sectional study, and fasting serum insulin levels, serum transferring levels and transferrin receptors were not evaluated in our study which could have provided with more precise information.

4. Conclusion

Increased levels of serum ferritin and serum iron were observed in diabetic patients in our study adding credibility to the hypothesis that iron metabolism might participate in the etiology of insulin resistance. Levels of serum ferritin and serum iron were not correlating with the glycemic control of diabetic patients (HbA1c) and according to our study hence could not be used for the same. Serum ferritin and serum iron levels were not correlating with the duration of diabetes. Serum ferritin and serum iron levels were correlating with the increasing number of complications of diabetes and hence, support the proposed role of iron induced tissue damage in the development of complications. Serum ferritin and serum iron levels were correlating with the use of regular treatment. However, no significant association was found between serum ferritin and serum iron levels and type of treatment taken and hence the same could not be used to establish the effectiveness of the type of treatment given. Therefore, it is suggested that Serum ferritin and iron could be added to routine evaluation of Diabetes Mellitus and prediabetes; this would help us identify a subgroup of individuals at risk of iron related tissue damage, in whom further investigations and therapeutic options may be appropriate. Reduced dietary intake of iron especially in individuals with additional risk factors for type2 diabetes may be advisable. The indiscriminate use of iron preparations should be avoided. Measures to reduce the iron load (like frequent blood donation) may help prevent the development of diabetes in those at risk and may also help retard the progression of tissue damage in diabetic patients.

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