RESEARCH ARTICLE

Association of Hepcidin levels in Type 2 Diabetes Mellitus treated with metformin or combined anti-diabetic agents in Pakistani population

Tabinda Najeeb[1], Mohammad Saleh Soomro (late)[2], Asher Fawwad[3], Nazish Waris[4], Ruqaya Nangrejo[5], Iftikhar Ahmed Siddiqui[6], Qamar Aziz[7], Abdul Basit[8]

Abstract

Objective: To evaluate the impact of hepcidin and ferritin in pathogenesis and prognosis of type 2 diabetes mellitus subjects taking only metformin or combined anti-glycaemic agents.

Methods: The observational case-control study was conducted at the Department of Physiology, Baqai Medical University, Karachi, from August 2019 to October 2020, and comprised subjects from both genders who categorised into equal groups as non-diabetic controls, newly-diagnosed type 2 diabetes mellitus patients without any treatment, type 2 diabetes mellitus patients with exposure to metformin only, type 2 diabetes mellitus patients taking oral hypoglycaemic agents along with metformin, type 2 diabetes mellitus patients taking only insulin, and type 2 diabetes mellitus patients taking insulin and oral hypoglycaemic agents. Fasting plasma glucose was determined using glucose oxidase-peroxidase method, glycated haemoglobin by high performance liquid chromatography, high-density lipoprotein and low-density lipoprotein by direct methods, cholesterol by cholesterol oxidase phenol 4-amino antipyrine peroxidase and triglycerides by glycerol phosphate oxidase-phenol 4-amino antipyrine peroxidase method. Serum levels of ferritin, insulin and hepcidin were evaluated using Enzyme-linked immunosorbent assay. Insulin resistance was assessed using homeostasis model assessment for insulin resistance. Data was analysed using SPSS 21.

Results: Of the 300 subjects, there were 50(16.66%) in each of the 6 groups. Overall, there were 144(48%) males and 155(51.66%) females. The mean age was significantly lower in the control group 34.72±7.87 compared to all the diabetic groups ($p<0.05$), and the same was the case with respect to all the parameters ($p<0.05$) except high-density lipoprotein ($p>0.05$). Besides, hepcidin level was significantly higher in the control group ($p<0.05$). Ferritin levels were significantly increased in newly-diagnosed T2DM subjects compared to the controls ($p<0.05$) while all other groups showed decreased ferritin levels ($p<0.05$). Hepcidin gave inverse correlation with glycated haemoglobin only in diabetics taking only metformin ($r = -0.27$, $p=0.05$).

Conclusion: Anti-diabetes drugs not only addressed type 2 diabetes mellitus, but also reduced levels of ferritin and hepcidin that are found to play a role in diabetes development.

Keywords: Ferritin, Hepcidin, Insulin, Oral hypoglycaemic agents, T2DM. (JPMA 73: 313; 2023)

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Introduction

Type 2 diabetes mellitus (T2DM) is a metabolic disorder reaching epidemic proportion globally. As per the International Diabetes Federation (IDF) in 2019, there were 463 million diagnosed cases of diabetes worldwide, which was predicted to increase up to 578 million in 2030 and 700 million in 2045.[1] In Pakistan, the prevalence of T2DM was estimated as 26.3 % by the second National Diabetes Survey of Pakistan (2016-17).[2]

Hepcidin hormone works for iron reabsorption across epithelial membrane of the gastrointestinal tract (GIT) and it also recycles the metal element in red blood cells (RBCs), placenta and macrophages.[3] Hepcidin plays its role by binding with ferroportin (FPN) receptor, which is the only transporter of iron in the body, and then causes the internalisation, inactivation and degradation of the exporter.[4] Hepcidin levels are declined when body needs more iron in circulation because diminished concentration of hepcidin results in the release of stored iron, and enhances iron absorption through gastric epithelium.[5]

Elevated concentration of hepcidin are found in some T2DM subjects that play important role in inflammation, overweight, obesity and chronic kidney impairment.[6] However, low levels of hepcidin in T2DM is not yet fully understood, even though it might play a key role in insulin resistance (IR).[7] It was documented that insulin may affect hepcidin synthesis directly by increasing expression of signal transducers and activators of transcription 3 (STAT3)

[1,2,5,7]Department of Physiology, Baqai Medical University, Karachi, Pakistan; [3]Department of Biochemistry, Baqai Institute of Diabetology and Endocrinology, Baqai Medical University, Karachi, Pakistan; [4,8]Baqai Institute of Diabetology and Endocrinology, Baqai Medical University, Karachi, Pakistan; [6]Department of Biochemistry, Baqai Medical University, Karachi, Pakistan.

Correspondence: Tabinda Najeeb. e-mail: tabindahansari@gmail.com

ORCID ID. 0000-0003-1834-8550

protein and deoxyribonucleic acid (DNA) binding activity in hepatocytes which explains deficient hepcidin concentration in T2DM.[8] Inappropriate levels of hepcidin accompanied with IR might lead to T2DM because of greater degradation of beta cells of pancreas due to iron overload.[9]

Metformin is a synthetic oral anti-diabetic drug clinically used as a first line treatment for diabetes across all age groups.[10,11] The association of metformin with hepcidin is still unclear as one study mentioned highly increased hepcidin levels with greater use of metformin,[11] while another study observed decreased hepcidin concentration with the intervention of metformin for lesser duration.[7] Though metformin is demonstrated to suppress the hepcidin production via the inhibition of STAT3 protein through adenosine monophosphate (AMP)-activated protein kinase (AMPK).[12]

To the best of our knowledge, no study in Pakistani population has explored the combined role of ferritin and hepcidin concentrations in T2DM with metformin and oral hypoglycaemic agents (OHAs). The current study was planned to fill the gap by analysing the effect of ferritin and hepcidin levels in T2DM subjects with exposure to metformin only, and/or in combination with other anti-glycaemic therapies in newly diagnosed and known diagnosed T2DM subjects.

Subjects and Methods

The observational case-control study was conducted at the Department of Physiology, Baqai Medical University (BMU), Karachi, from August 2019 to October 2020. After approval from the institutional ethics review committee, the sample size was calculated using OpenEpi calculator[13] with 80% power of test, 5% two-sided level of significance, and ratio of sample size 1. Mean hepcidin values for control and T2DM subjects were taken from an earlier study.[9] The sample was raised using consecutive sampling technique from the BMU's Baqai Institute of Diabetology and Endocrinology.

Those included were adult diabetics of either gender aged 18-70 years. Healthy controls matched for age and gender were enrolled from the community. Subjects with acute cardiac diseases, liver abnormalities, acute infections, autoimmune diseases, pregnancy, type 1 diabetes mellitus, pre-diabetes and subjects taking glucocorticoids were excluded.

After taking informed consent, the subjects were divided into 6 equal groups. Group-1comprised healthy controls; Group-2 had newly-diagnosed T2DM subjects without any treatment; Group-3 had T2DM subjects with exposure to metformin only; Group-4 comprised T2DM subjects with exposure to other OHAs in addition to metformin; Group-5 subjects were taking only insulin treatment; and Group-6 subjects were taking both insulin and OHAs.

Data was collected using a customised pre-designed proforma with demographic, anthropometric, socio-economic and medication history. From each participant, blood sample was drawn after 10-12 hours of fasting to estimate the level of serum hepcidin, serum insulin, serum ferritin, lipid profile, fasting plasma glucose (FPG) and glycated haemoglobin (HbA1c).

FPG ≥126mg/dL and/or HbA1c >6.5% confirmed T2DM. Duration of newly-diagnosed T2DM subjects was not >365 days. Individuals with FPG <100mg/dL were in controlGroup-1.[14]

HbA1c was assessed by using high performance liquid chromatography (HPLC),[15] FPG by glucose oxidase-peroxidase (GOD-POD) method, total cholesterol (TC) levels by cholesterol oxidase phenol 4-amino antipyrine peroxidase (CHOD-PAP) method, triglycerides (TG) by glycerol phosphate oxidase phenol 4-amino antipyrine peroxidase (GPO-PAP) method by enzymatic hydrolysis.[16] High-density lipoprotein (HDL) and low-density lipoprotein (LDL) were estimated by direct methods. Serum ferritin concentration was quantitatively determined by using enzyme-linked immunosorbent assay (ELISA) kit, serum insulin was estimated by using insulin immunoassay test kit (DiaMetra, Italy). Hepcidin concentration was measured on ELISA by using Human Hepcidin 25 (Hepc-25) ELISA kit[17] (Bioassay Technology Laboratory, United Kingdom). The absorbance/results were read with the help of ELISA machine (DR-200Bs Microplate Reader, Diatek, United States), calibrated as per the instructions on the kit. the Homeostasis model assessment for insulin resistance (HOMA- IR) index was calculated using the following equation:

$$\text{HOMA-IR} = \frac{(\text{Fasting insulin level} \times \text{Fasting blood glucose level})}{22.5}$$

Data was analysed using SPSS 21. Data was presented as mean±standard deviation (SD) for normal distribution and median (interquartile range [IQR]) for non-normally distributed parameters. Data normality was checked using Shapiro Wilk test. One-way analysis of variance (ANOVA)/Kruskal-Wallis test was used to observe significant difference in different T2DMgroups. Pearson/Spearman correlation was used to find the association between the studied variables. Linear regression was employed to assess the effect of biochemical parameters on hepcidin. $P<0.05$ was considered statistically significant.

Table-1: Comparison of demographic, glycaemic and clinical parameters.

Parameters	Non-Diabetic Control	T2DM Subjects					*p*-value
	Group 1 (n=50)	**Group 2** (n=50)	**Group 3** (n=50)	**Group 4** (n=50)	**Group 5** (n=50)	**Group 6** (n=50)	
Males	24	22	21	31	24	22-	
Females	26	28	29	19	26	28	
Age (years)	34.72±7.87	43.46±9.98	49.08±10.02	50.40±9.12	53.22±10.94	50.10±9.17	< 0.001
BMI (Kg/m^2)	24.31±4.38	30.19±6.40	29.21±4.81	29.79±4.54	28.95±4.57	30.41±5.23	< 0.001
FPG (mg/dl)	90.42±8.46	164.18±68.68	170.46±59.25	186.0±78.55	171.76±83.46	178.70±56.12	< 0.001
HbA1c (%)	5.06 ± 0.38	9.22±2.66	8.74±2.06	9.50±2.96	9.70±2.06	9.16±1.64	< 0.001
Total Cholesterol (mg/dl)	168.62±34.46	183.57±42.97	187.68±47.57	184.70±43.70	174.44±43.70	161.78±51.44	0.023
Triglycerides (mg/dl)	113.74±55.07	155.3±94.46	150.80±70.07	209.64±103.04	174.82±92.31	196.50±108.5	< 0.001
High Density Lipoprotein (mg/dl)	31.04±5.42	31.56±8.29	32.04±6.88	30.14±6.50	30.94±7.64	32.62±7.33	0.572
Low Density Lipoprotein (mg/dl)	108.95±29.81	113.12±25.17	113.30±32.06	115.55±31.21	101.52±38.35	88.90±31.61	0.001
Serum Insulin (ng/ml)	13.23±5.88	18.86±11.19	16.41±8.32	16.35±10.1	23.74±12.17	24.89±11.70	< 0.001
Insulin Resistance	2.97±1.37	7.41±5.11	6.75±3.54	7.94±6.23	9.62±6.38	10.89±7.89	< 0.001
Serum Ferritin (ng/ml)	70.95±64.96	82.84±66.87	63.93±47.57	64.26±41.91	51.51±35.46	41.62±31.92	0.001
Serum Hepcidin (ng/ml)	702.80 (926.50- 644.77)	630.20 (747–313.35)	430.45 (951.75–96.49)	328.05 (695.47–109.12)	573.0 (731.80–277.10)	592.95 (1237.75–98.99)	0.003

Data presented as Mean ± standard deviation (SD) or median (interquartile range [IQR]); T2DM: Type 2 diabetes mellitus, BMI: Body mass index, FPG: Fasting plasma glucose, HbA1c: Glycated haemoglobin, Group 1: Control (non-diabetic healthy subjects), Group 2: Newly diagnosed T2DM without any treatment, Group 3: T2DM subjects taking metformin only: Group 4: T2DM subjects taking metformin and other oral hypoglycaemic agents [OHAs]; Group 5: T2DM subjects taking insulin only: Group 6: T2DM subjects taking insulin and other OHAs.

Results

Of the 300 subjects, there were 50(16.66%) in each of the 6 groups. Overall, there were 144(48%) males and 155(51.66%) females. The mean age was significantly lower in the control group 34.72±7.87 compared to all the diabetic groups (p<0.05), and the same was the case with respect to all the parameters (p<0.05) except HDL (p>0.05). Besides, hepcidin level was significantly higher in the control group (p<0.05). Ferritin levels were significantly increased in newly-diagnosed T2DM subjects compared to the controls (p<0.05) while all other groups showed decreased ferritin levels (p<0.05) (able 1).

Correlation analysis of clinical parameters across all the 6 groups was tabulated (Table 2).

Univariate linear regression showed that HbA1c affected hepcidin levels only in Group-3(R2 = 0.066) explaining that 6.6% variability in hepcidin concentration is due to HbA1c, and the fact that regression coefficient (B) was negative demonstrated an inverse relationship between HbA1c and hepcidin levels. In Group-6, IR predicted significant effect, explaining 7.3% variability in hepcidin values, negative B value indicated that one-unit increase in IR in Group-6 decreased hepcidin levels by 21.75 and vice versa (Table 3).

Effects of altered hepcidin (Table 4) and ferritin (Table 5) concentrations on HbA1c levels in all the groups were also studied.

Unlike hepcidin, increased HbA1c was found with higher serum ferritin concentration, but beyond 150ng/ml of ferritin, HbA1c began to drop again.

Discussion

Overall, the current study found low levels of hepcidin in all T2DM groups. In newly-diagnosed T2DM subjects without any anti-diabetes treatment relatively higher hepcidin levels were found, but the level decreased in groups taking metformin alone and combined therapy of metformin with OHAs. Again, hepcidin level increased in DM groups taking insulin alone and combined insulin with OHAs, indicating some contribution of hepcidin to T2DM pathogenesis or prognosis.[11] This reflects the effects of hypoglycaemic drugs on hepcidin levels. It is observed that insulin administration potentiates the secretion of hepcidin via STAT3 protein which is a crucial modulator for hepcidin transcription.[18,19] Another mechanism that links hepcidin secretion with insulin is the glucose stimulation. Hepcidin can also be produced extra-hepatically from beta cells of pancreas, therefore when glucose stimulates insulin secretion, it simultaneously secretes hepcidin from same granules that may act as paracrine fashion.[20,21] Thus, the oral anti-diabetic agents that decrease the glucose levels in plasma might be responsible for concomitant decrease in hepcidin concentrations. Some studies explored the relationship between hepcidin levels and T2DM, but the findings are inconsistent and vague as no exact association was found.[22] The current results are consistent with a study reporting significantly lower levels of hepcidin in T2DM subjects compared to the healthy control.[7] Whereas in newly diagnosed diabetes subjects, no exogenous insulin and/or OHAs are liable for decreased hepcidin concentration instead the increased ferritin/iron levels are thought to be the reason behind T2DM development.

Ferritin is the complex protein that plays a role in the

Table-2: Correlation of biochemical parameters.

Groups	Characteristics		FPG	HbA1c	Insulin	I.R	Ferritin	Hepcidin
Group 1	FPG	r (p)	1.00	0.227 (0.158)	0.326 (0.040*)	0.485 (0.002*)	-0.184 (0.257)	0.066 (0.684)
	HbA1c	r (p)		1.00	0.284 (0.076)	0.304 (0.05*)	-0.400 (0.01*)	0.028 (0.863)
	Insulin	r (p)			1.00	0.980(< 0.001*)	-0.082(0.614)	-0.165(0.308)
	I.R.	r (p)				1.00	-0.096 (0.554)	-0.127 (0.436)
	Ferritin	r (p)					1.00	0.261(0.104)
	Hepcidin	r (p)						1.00
Group 2	FPG	r (p)	1.00	0.783(0.000*)	-0.800(0.623)	0.530(< 0.001*)	0.071(0.665)	0.030(0.852)
	HbA1c	r (p)		1.00	0.267 (0.096)	0.207(0.201)	0.04(0.785)	-0.028(0.866)
	Insulin	r (p)			1.00	0.74(< 0.001*)	0.186(0.249)	0.266(0.097)
	I.R.	r (p)				1.00	0.106(0.516)	0.114(0.483)
	Ferritin	r (p)					1.00	0.034(0.836)
	Hepcidin	r (p)						1.00
Group 3	FPG	r (p)	1.00	0.661(0.000*)	-0.102(0.481)	0.447(0.001*)	-0.036(0.801)	-0.199(0.167)
	HbA1c	r (p)		1.00	-0.178(0.217)	0.241(0.092)	-0.028(0.849)	-0.270(0.05*)
	Insulin	r (p)			1.00	0.81(< 0.001*)	0.126(0.382)	-0.037(0.796)
	I.R.	r (p)				1.00	0.051(0.723)	-0.252(0.077)
	Ferritin	r (p)					1.00	0.142(0.324)
	Hepcidin	r (p)						1.00
Group 4	FPG	r (p)	1.00	0.748(0.001*)	0.049(0.763)	0.478(0.002*)	-0.013(0.937)	-0.292(0.068)
	HbA1c	r (p)		1.00	-0.324(0.041*)	-0.11(0.947)	0.337(0.034*)	-0.092(0.573)
	Insulin	r (p)			1.00	0.81(< 0.001*)	-0.282(0.078)	-0.124(0.446)
	I.R.	r (p)				1.00	-0.202(0.212)	-0.049(0.762)
	Ferritin	r (p)					1.00	0.169(0.297)
	Hepcidin	r (p)						1.00
Group 5	FPG	r (p)	1.00	0.111(0.489)	-0.185(0.247)	0.55(< 0.001*)	0.175(0.274)	-0.049(0.763)
	HbA1c	r (p)		1.00	0.170(0.289)	0.260(0.101)	0.113(0.481)	-0.032(0.845)
	Insulin	r (p)			1.00	0.66(< 0.001*)	0.278(0.078)	-0.144(0.371)
	I.R.	r (p)				1.00	0.370(0.017*)	-0.092(0.569)
	Ferritin	r (p)					1.00	-0.065(0.685)
	Hepcidin	r (p)						1.00
Group 6	FPG	r (p)	1.00	0.426(0.006*)	0.036(0.823)	0.453(0.003*)	-0.029(0.859)	-0.238(0.138)
	HbA1c	r (p)		1.00	-0.067(0.680)	0.273(0.088)	-0.047(0.773)	-0.0274(0.87)
	Insulin	r (p)			1.00	0.87(< 0.001*)	-0.159(0.326)	-0.185(0.252)
	I.R.	r (p)				1.00	-0.147(0.366)	-0.290(0.070)
	Ferritin	r (p)					1.00	0.183(0.259)
	Hepcidin	r (p)						1.00

Data presented as r =correlation coefficient; p<0.05 was considered statistically significant; FPG: Fasting plasma glucose, HbA1c: Glycated haemoglobin, IR: insulin resistance, Group 1: Control (non-diabetic healthy subjects), Group 2: Newly diagnosed T2DM without any treatment, Group 3: T2DM subjects taking metformin only: Group 4: T2DM subjects taking metformin and other oral hypoglycaemic agents [OHAs]; Group 5: T2DM subjects taking insulin only: Group 6: T2DM subjects taking insulin and other OHAs.

storage of excess iron in non-toxic and soluble form. Increased amount of ferritin in cells may lead to IR and, ultimately, destruction of beta cells. Hyperinsulinaemia prior to development of full-blown DM may be attributed to higher levels of ferritin.[23] The current study observed similar findings in Group-2 in line with an earlier study.[24] In other T2DM groups, a decline in ferritin concentration was witnessed with significant decrease in groups 5 and 6. The findings are consistent with literature.[25] The exact molecular mechanism for this difference is not known yet.

Higher ferritin levels are observed in uncontrolled DM subjects despite the use of anti-diabetes agents when compared with controlled DM and healthy subjects.[26] A recent study on rodent model observed that iron chelator desferroxamine (DFO) not only decreases iron levels but also improves insulin levels and sensitivity, increased expression of glucose transporter 1 (GLUT1) and GLUT4 in adipose tissues.[27]

The association of metformin with hepcidin is still unclear.[5] Literature found altered hepcidin levels in prediabetes state.[28]

When different groups of diabetes were compared, it was found that Group-3 had lesser mean values of HbA1c compared to other diabetes groups, which is consistent with an earlier study.[29]

It is worth noting that Group-3 in the current study had lowest IR among all the diabetes groups. Lin et al. reported decreased IR in DM patients receiving insulin alone and combined OHA-plus-insulin in two different groups when compared to newly diagnosed diabetes subjects, which is

Table-3: Regression analysis assessing factors associated with hepcidin.

Groups		Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Age (years)	B	-2.865	8.690	0.051	6.367	-0.370	-2.636
	R^2	0.014	0.058	0.061	0.025	0.000	0.001
	p-value	0.553	0.126	0.151	0.379	0.931	0.855
Body mass index (BMI) (Kg/m^2)	B	-9.621	-5.949	-0.060	21.746	-5.029	-22.221
	R^2	00.061	0.010	0.019	0.081	0.007	0.035
	p-value	0.181	0.526	0.251	0.086	0.520	0.223
Fasting plasma glucose (mg/dl)	B	1.976	-0.599	-2.383	-0.736	-0.189	-2.961
	R2	0.006	0.015	0.039	0.034	0.002	0.057
	p-value	0.637	0.432	0.197	0.224	0.791	0.165
HbA1c (%)	B	13.673	1.021	-88.353	-16.729	-4.446	-115.469
	R^2	0.001	0.000	0.066	0.025	0.001	0.075
	p-value	0.839	0.967	0.042*	0.202	0.841	0.087
Insulin (ng/ml)	B	-5.470	9.065	-3.242	4.580	-3.482	-5.320
	R^2	0.030	0.071	0.001	0.018	0.021	0.018
	p-value	0.231	0.113	0.832	0.378	0.347	0.320
Insulin resistance (ng/ml)	B	-23.70	7.920	-50.542	-2.122	-4.391	-21.756
	R^2	0.029	0.013	0.064	0.002	0.008	0.073
	p-value	0.245	0.529	0.136	0.751	0.461	0.048*
Ferritin (ng/ml)	B	0.702	0.172	2.127	0.588	-0.56	4.212
	R^2	0.068	0.001	0.020	0.006	0.004	0.033
	p-value	0.075	0.815	0.333	0.685	0.624	0.338

B: Regression coefficient, R2: Linear regression, HbA1c: Glycated haemoglobin, Group 1: Control (non-diabetic healthy subjects), Group 2: Newly diagnosed T2DM without any treatment, Group 3: T2DM subjects taking metformin only: Group 4: T2DM subjects taking metformin and other oral hypoglycaemic agents [OHAs]; Group 5: T2DM subjects taking insulin only: Group 6: T2DM subjects taking insulin and other OHAs. P<0.05 was considered statistically significant.

Table-4: Comparison of altered hepcidin levels on HbA1c in cases and controls.

Altered Hepcidin Levels (ng/ml)	**HbA1c (%)** (overall)	**HbA1c (%)** (Control)	**HbA1c (%)** (Cases)
<250	9.26±2.80	-	9.14±1.99
250–500	8.56±2.42	4.40±0.56	8.55±1.43
501–750	7.91±2.96	5.0±0.29	8.60±1.74
751–1000	7.14±2.24	4.86±0.35	9.16±1.99
>1000	8.91±2.79	5.20±0.17	9.53±2.42
***p*-value**	0.002	0.038	0.132

HbA1c: Glycated haemoglobin.

Table-5: Comparison of altered ferritin levels on HbA1c in cases and controls.

Altered Hepcidin Levels (ng/ml)	**HbA1c (%)** (overall)	**HbA1c (%)** (Control)	**HbA1c (%)** (Cases)
<30	8.13±2.33	5.17±0.32	8.86±2.02
30 – 70	8.69±2.44	5.05±0.41	9.18±2.16
71 – 100	9.07±2.38	5.16±0.32	9.50±2.12
101 – 150	9.43±3.72	5.12±0.38	10.51±3.33
>150	7.15±2.58	4.71±0.39	8.55±2.27
***p*-value**	0.004	0.050	0.011

HbA1c: Glycated haemoglobin; Ferritin <30 = iron deficiency, 30-150 = sufficient/good iron stores, >150 = iron overload.

in contrast to the current findings.[30]

Cholesterol and LDL levels were found to be the lowest in Group-6 compared to control and other diabetes groups. This is consistent with a previous study.[31]

The present study did not find association between ferritin and hepcidin in any group on drug basis, nor altered hepcidin reflect any impact on the control of DM. It was observed that by increasing the ferritin levels, HbA1c also raised significantly, but no significant difference on glycated Hb was seen with different hepcidin values. This reflects the fact that though levels are found decreased in T2DM participants, hepcidin is not the culprit behind the change in glycated Hb values in DM subjects in present study. Significant inverse association of ferritin with hepcidin was seen only in iron-deficient group, while iron-sufficient and iron-overload groups showed no association with ferritin. Statistically significant difference for ferritin was seen on HbA1c levels, and weak association of ferritin with HbA1c was observed, suggesting that iron overload could be involve in development of DM, or DM can bidirectionally elevate ferritin levels due to low-grade inflammation associated with it. But these findings need further validation through a larger dataset as in present study, only a small number of diabetic subjects (6%) had iron overload, while 38% and 31.2 % control and diabetic subjects, respectively, were-iron deficient in the current study.

The present study has limitations, too, as the effects of OHAs other than metformin was not assessed individually. Follow-up based prospective cohort studies with larger sample sizes are recommended to validate the findings of the current study. Also, the dose of different anti-glycaemic agents with their effects on these clinical variables should be studied to observe the diabetic prognosis and complications.

Conclusion

Decreased hepcidin levels in all diabetic groups underlined its role in T2DM pathogenesis. Increased ferritin levels in specific groups signify the role of iron in the development of T2DM.

Disclaimer: The text is based on a Ph.D. thesis.

Conflict of Interest: None.

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