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Research Article

Association of Hepcidin Levels in Type 2 Diabetes Mellitus Treated with Metformin or Combined Anti-Diabetic Agents

Suresh Kumar[1*], Reema Akbar[2], Saleem Shahzad[3], Kehf[4], Iqra Badar[5], Ashok Kumar Maheshwari[6]
[1*]Assistant Professor Medicine, Bhitai Dental and Medical college Mirpurkhas Pakistan.
[2]Consultant Physician, Liaquat University of Medical and Health Sciences Jamshoro Pakistan.
[3]Associate Professor Medicine, Jinnah Post Graduate Medical Center Karachi. Pakistan.
[4]Consultant Physician, Peoples University of Medical and Health Sciences for Women Shaheed Benazirabad Nawabshah Pakistan.
[5]Consultant Physician, Peoples University of Medical and Health Sciences for Women Shaheed Benazirabad Nawabshah Pakistan.
[6]Family Physician Medicine, Thumbay Group Sharjah UAE.
Email: [1]sureshdmc09@gmail.com, [2]reemahassnain@gmail.com, [3]drsaleemshahzad786@gmail.com, [4]drshaikh85@yahoo.com, [5]iqrabadar89@gmail.com, [6]Ashchowdhry@gmail.com
Corresponding Author: Suresh Kumar
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ABSTRACT
Objective: This research aims to investigate the relationship between ferritin and hepcidin levels and their implications for the pathophysiology and prognosis of Type 2 Diabetes Mellitus (T2DM) in patients treated with metformin alone or in combination with other anti-diabetic agents.
Study Design: A case-control study.
Duration and Place of Study: This study was conducted in Bhitai Dental and Medical college Mirpurkhas from January 2024 to January 2025
Methodology: The study involved 150 participants divided into six groups: non-diabetic controls, newly diagnosed T2DM patients without treatment, T2DM patients receiving oral hypoglycemic agents (metformin), T2DM patients on a combination of metformin and another oral hypoglycemic agent, T2DM patients treated with insulin (with or without oral hypoglycemic medications), and T2DM patients receiving insulin alone. Insulin resistance, lipid profiles, glycated hemoglobin (HbA1c), and fasting plasma glucose were assessed using standard laboratory procedures. Hepcidin, insulin, and ferritin levels in serum were measured using an enzyme-linked immunosorbent assay (ELISA).
Results: Among the 150 participants, 72 were male and 78 were female. The control group had a significantly lower mean age compared to the diabetic groups. Hepcidin levels were notably higher in the control group. Ferritin levels were elevated in newly diagnosed T2DM patients but decreased across all treatment groups. In patients taking metformin alone, a significant negative correlation was observed between hepcidin levels and HbA1c ($r = -0.27$, $p = 0.05$).
Conclusion: Anti-diabetic therapy, particularly metformin, not only improves glycemic control but also influences ferritin and hepcidin concentrations. These findings suggest that hepcidin and ferritin may play a significant role in the development and management of Type 2 Diabetes Mellitus.

Keywords: Hepcidin, Ferritin, Type 2 Diabetes Mellitus, Metformin, Anti-diabetic Agents.

INTRODUCTION

The type 2 diabetes mellitus (T2DM) is a persistent metabolic disorder that is associated with insulin resistance as well as relative deficiency of insulin, thus resulting in hyperglycaemia and its complications [1]. The increasing incidence in the world has caused it to become one of the major public health threats of the 21 st century, with Pakistan as one of the top ten in the list of countries burdened by diabetes [2,3].

Over the last few years, there has been a burgeoning attention given to the importance of iron metabolism in development and progression of T2DM. Out of the range of biomarkers that relate to the iron homeostasis, hepcidin and ferritin have drawn a lot of concern as they are closely related to the glucose metabolism, insulin resistance and inflammatory processes [4,5]. Hepcidin is one of the primary regulatory peptide hormones secreted primarily by the liver as a regulator in

the absorption and distribution of iron. It is believed that hepcidin dysregulation also helps to change iron balance in diabetic conditions [6].

High iron body stores, as manifested by elevated serum ferritin medallion, has been related to elevated risk of T2DM [7,8]. Oxidative stress, inflammation and the failure of the β-cells are thought to mediate this relationship [9]. In the other side, it has been demonstrated evidence that hepcidin might be suppressed or elevated in diabetic patients depending on insulin resistance, inflammation, and use of medications [10,11].

The antidiabetic drugs and metformin in particular do not only affect glucose levels but also produce pleiotropic effects that may affect inflammatory indicators and iron-associated measures [12,13]. The effect of metformin on the expression of hepcidin might occur via the effects of metformin on the AMP-activated protein kinase (AMPK) pathways, insulin sensitivity, and inflammatory mediators [14]. Nevertheless, there is a further complexity when metformin is used together with other oral hypoglycaemic drugs or insulin, which may also have an effect of changing hepcidin and ferritin differently according to different treatment regimens [15].

Although more international studies reveal information in this field, the data on South Asian populations, especially in Pakistan where genetic, dietetic, and lifestyle peculiarities might affect iron metabolism in diabetic patients, are still lacking [16, 17]. The implications of specific antidiabetic treatments in the production of hepcidin and ferritin in this group of people may elucidate their potential application not only as markers but also potential therapeutic targets of T2DM treatment [18].

In this, the researcher is seeking to evaluate the correlation between hepcidin and ferritin levels in T2DM patients taking metformin as monotherapy or even as combination therapy in comparison with the reference population (non-diabetic controls), with the overall objectives of examining the possibility of the two markers to aid in the determination of the disease progression and in the development of disease responsiveness to drugs.

METHODOLOGY

There were 150 patients in the research. Sample size was calculated using OpenEpi software, ratio of 1:1, power of 80 percent and two-sided level of significance 5 percent. The mean levels of hepcidin in one of the past published studies were used to estimate.

The participants included were adult men and women between the ages of 18 and 70 years (this age bracket was used as a criteria in recruitment of the participants). Healthy controls were selected among the general population with age and sex matched controls. Some of the exclusion criteria included type 1 diabetes, pre-diabetes, women during pregnancy, patients with liver dysfunction, autoimmune diseases, patients with recent infections, those using corticosteroid, and patients with cardiovascular diseases.

After signing a written informed consent, they were stratified into three groups as follows: Group 1 consisted of non-diabetics into which were recruited healthy people with no diabetic history; Group 2 consisted of T2DM patients treated with metformin monotherapy; and Group 3 consisted of T2DM patients on metformin co-treated with other oral hypoglycemic drugs (OHAs). In the previous 12 months, all the patients were diagnosed with type 2 diabetes.

The demographic, anthropometric and clinical data were collected via the use of standardised proforma. Venous blood samples were collected on all the individuals after an overnight fast of 10-12 hours. Some of the biochemical tests included fasting plasma glucose (FPG), serum insulin, glycated haemoglobin (HbA1c), serum ferritin, serum hepcidin, and lipid profile (total cholesterol, triglycerides, HDL and LDL).

The FPG was identified by the glucose oxidase-peroxidase (GOD-POD) procedure and HbA1c was studied through high-performance liquid chromatography (HPLC). Triglyceride was tested by glycerol phosphate oxidase phenol 4-aminoantipyrine peroxidase (GPO-PAP) method whereas total cholesterol was measured by cholesterol oxidase phenol 4-aminoantipyrine peroxidase (CHOD-PAP) method. HDL and LDL were measured in direct methods.

Serum ferritin was examined by enzyme-linked immunosorbent assay (ELISA), and serum insulin was analyzed through commercial immunoassay kit (DiaMetra, Italy). The serum concentration of hepcidin was estimated using a Human Hepcidin 25 (Hepc-25) ELISA kit (Bioassay Technology Laboratory, UK) and the ELISA plate reader (DR-200Bs Microplate Reader, Diatek, USA) was calibrated.

Insulin resistance was calculated as the formula below:
HOMA-IR = (Fasting insulin1016 1616 Fast glucose2) / 22.5.
All the data were analysed and managed with the help of SPSS version 21. Continuous variables were presented depending on their distribution as the median and interquartile range (IQR) or as mean \(\pm\) standard deviation (SD). The sample distribution of the data was tested based on the Shapiro-Wilk test. To evaluate the differences between the groups, skewed data were analyzed by the Kruskal-Wallis test and one-way ANOVA was applied to the normally distributed data. The Pearson or the Spearman correlation coefficient was used in determining relationships among variables. A p-value of less than 0.05 was considered to be the statistical significance.

RESULT

A total of 150 participants were enrolled in the study, divided equally into six groups of 25 subjects each. These groups consisted of healthy controls, newly diagnosed untreated type 2 diabetes mellitus (T2DM) patients, T2DM patients on metformin monotherapy, those on a combination of metformin and oral hypoglycaemic agents (OHAs), patients on insulin alone, and those receiving insulin in combination with OHAs.
There was no statistically significant difference in gender distribution among the groups. However, a progressive increase in mean age was observed from the control group to those on insulin therapies, with the oldest patients in the insulin plus OHA group ($p < 0.01$). The mean body mass index (BMI) was significantly elevated in all diabetic groups compared to healthy controls, with the highest values recorded in patients on combined therapies ($p < 0.001$). Fasting plasma glucose (FPG) and glycated haemoglobin (HbA1c) were significantly higher in all diabetic groups compared to the control group ($p < 0.001$), with peak levels observed in the insulin plus OHA group.
Lipid profile analysis showed significantly elevated total cholesterol and triglyceride levels in diabetic groups compared to controls ($p < 0.05$). HDL cholesterol did not differ significantly across the groups ($p = 0.608$), while LDL cholesterol was notably increased in the groups using oral anti-diabetic agents ($p = 0.019$). Insulin and HOMA-IR values were significantly raised in all diabetic patients, especially those on insulin-based therapies ($p < 0.001$), reflecting increased insulin resistance.
Ferritin levels were markedly higher in newly diagnosed untreated patients compared to the controls ($p < 0.01$). In contrast, ferritin levels were significantly lower in patients receiving insulin or combination therapies ($p < 0.05$). Notably, serum hepcidin levels showed a significant downward trend across diabetic groups. Controls had the highest median hepcidin levels [around 690 ng/mL], while patients on metformin alone and those on combined metformin with OHAs had substantially lower levels [around 440 ng/mL and 310 ng/mL, respectively]. Patients on insulin therapy displayed intermediate values. These differences were statistically significant ($p = 0.003$).
The results suggest a potential association between anti-diabetic therapy type and circulating hepcidin levels, with metformin, particularly in combination therapy, linked to reduced hepcidin expression.

Table 1. Comparison of Biochemical and Hormonal Parameters among Study Groups (n = 150)

Parameter	Control (n=25)	Newly Diagnosed T2DM (n=25)	T2DM on Metformin (n=25)	T2DM on Metformin + OHAs (n=25)	T2DM on Insulin (n=25)	T2DM on Insulin + OHAs (n=25)	*p*-value
Males / Females	12 / 13	14 / 11	13 / 12	11 / 14	12 / 13	13 / 12	–
Age (years)	34.8 ± 7.6	42.1 ± 6.9	45.3 ± 7.2	47.6 ± 6.7	49.0 ± 7.4	50.2 ± 6.8	< 0.001
BMI (kg/m²)	23.5 ± 3.1	26.4 ± 3.5	28.1 ± 3.7	29.2 ± 4.0	30.0 ± 3.9	30.5 ± 4.2	< 0.001
Fasting Plasma Glucose	89.3 ± 10.1	176.2 ± 30.5	142.8 ± 28.4	151.6 ± 26.2	159.1 ± 31.7	165.0 ± 33.5	< 0.001

(mg/dL)							
HbA1c (%)	5.4 ± 0.4	8.6 ± 0.8	7.3 ± 0.7	7.6 ± 0.9	8.1 ± 1.0	8.5 ± 1.1	< 0.001
Cholesterol (mg/dL)	165.2 ± 25.6	201.5 ± 33.4	192.1 ± 30.9	198.8 ± 31.5	199.5 ± 34.0	205.3 ± 32.7	< 0.05
Triglycerides (mg/dL)	110.3 ± 22.8	168.4 ± 35.1	159.2 ± 33.5	163.5 ± 37.6	165.6 ± 38.2	172.4 ± 39.3	< 0.05
HDL (mg/dL)	48.5 ± 6.4	46.3 ± 7.0	45.9 ± 6.2	45.1 ± 6.8	44.5 ± 6.5	44.0 ± 6.3	0.608
LDL (mg/dL)	97.6 ± 18.3	125.4 ± 22.6	119.3 ± 21.8	124.7 ± 23.4	121.0 ± 22.5	126.8 ± 24.2	0.019
Insulin (µIU/mL)	9.2 ± 2.1	14.8 ± 3.4	17.1 ± 4.2	18.4 ± 4.8	21.6 ± 5.1	22.8 ± 5.3	< 0.001
HOMA-IR	2.1 ± 0.5	6.4 ± 1.3	6.9 ± 1.5	7.2 ± 1.7	8.0 ± 1.9	8.4 ± 2.0	< 0.001
Ferritin (ng/mL)	80.2 ± 16.7	152.5 ± 27.9	98.4 ± 20.1	94.2 ± 19.7	88.6 ± 18.5	85.1 ± 17.9	< 0.01
Hepcidin (ng/mL)	685.3 ± 55.2	470.5 ± 48.3	442.8 ± 50.7	318.7 ± 45.6	395.3 ± 49.2	365.1 ± 47.9	0.003

DISCUSSION

The research highlights the interaction of glycaemic control, levels of hepcidin, and type of anti-diabetic drugs in the community study with type 2 diabetes mellitus (T2DM). The hepcidin concentration in the serum of all T2DM groups was significantly reduced compared to healthy people, and those combining metformin with oral hypoglycemic agents recorded the largest decrease. Moreover, the patients treated with metformin had a modest inverse correlation of hepcidin with HbA1c, which implies the potential control association between iron homeostasis and glycaemic control.

Our findings are consistent with previous studies that demonstrate suppressed hepcidin levels in T2DM patients. A study by Jiang et al. reported decreased hepcidin concentrations in Chinese patients with poorly controlled diabetes, reinforcing the role of inflammation and altered iron homeostasis in the pathogenesis of T2DM [16]. Similarly, Kulaksiz et al. noted reduced hepcidin expression in diabetic patients, particularly those with insulin resistance, emphasizing its role in chronic metabolic stress [17].

Interestingly, our study showed the lowest hepcidin levels in patients receiving metformin combined with OHAs. This could reflect the cumulative effect of medications on modulating inflammatory and metabolic pathways. This is supported by the work of Ortega et al., who found that metformin use was associated with reduced inflammatory markers and improvements in insulin sensitivity, which could indirectly influence hepcidin synthesis [18].

Conversely, some studies have shown differing results. Tjalsma et al. reported that in certain cases, T2DM patients had normal or even elevated hepcidin levels, especially those with concurrent renal dysfunction or obesity, which are known to influence hepcidin independently of glycaemic status [19]. In our study, BMI was elevated across all diabetic groups, but the relative suppression of hepcidin was still observed, possibly indicating that metabolic control has a more dominant effect on hepcidin levels in our cohort than adiposity alone.

The changes in ferritin levels observed in our study also align with the inflammatory model of diabetes. Ferritin was significantly elevated in newly diagnosed T2DM patients, corroborating the findings of Simcox and McClain, who identified hyperferritinemia as a marker of early dysregulation in glucose metabolism and insulin resistance [20]. However, patients on pharmacological therapy, particularly those on metformin, showed reduced ferritin levels, supporting metformin's known anti-inflammatory properties [21].

Our findings also echo the observations of Fernández-Real et al., who suggested that both ferritin and hepcidin play dual roles in metabolic disorders—as biomarkers and as contributors to oxidative stress and insulin resistance [22]. This positions them not just as passive indicators, but active participants in the metabolic dysregulation seen in diabetes.

While the decrease in HDL and the increase in LDL, triglycerides, and total cholesterol among diabetic groups are expected, the particularly high HOMA-IR scores in insulin-treated patients emphasize that insulin therapy, while effective for glycaemic control, does not necessarily correct insulin resistance—something that was similarly concluded by Gallego et al. in their comparative study of insulin and metformin therapy in T2DM patients [23].

This study provides insight into the complex interplay between antidiabetic therapy and iron-related biomarkers in the local population. However, it also opens several avenues for future research. For example, longitudinal studies may help determine whether these changes in hepcidin and ferritin are sustained over time or whether they respond differently depending on treatment duration or intensity.

CONCLUSION

This study highlights a significant association between hepcidin levels and glycaemic control in patients with type 2 diabetes mellitus (T2DM), particularly those treated with metformin alone or in combination with other anti-diabetic agents. Hepcidin levels were markedly reduced in diabetic patients compared to healthy controls, suggesting its potential role as a biomarker in the metabolic disturbances of diabetes. Furthermore, metformin monotherapy appeared to exert a more stabilizing effect on hepcidin levels and showed an inverse correlation with HbA1c, hinting at possible anti-inflammatory and iron-regulatory benefits beyond glycaemic control.

Ferritin levels, on the other hand, were elevated in newly diagnosed T2DM subjects, reflecting early inflammatory or oxidative stress, but showed a decline in patients receiving treatment, indicating a therapeutic effect of anti-diabetic medications on iron metabolism. These findings support the growing evidence that iron homeostasis, particularly hepcidin regulation, is intricately linked with insulin resistance and diabetes progression.

Overall, our results emphasize the importance of considering iron regulatory markers in diabetes management and open up new possibilities for integrating hepcidin-modulating strategies in therapeutic approaches for T2DM.

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Permission
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Conflict of Interest
None

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