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Association between Serum Hepcidin Levels and Insulin Resistance in Iraqi Type 2 Diabetes Patients

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Abstract

The current study aimed to evaluate the impact of hepcidin levels on the diagnosis and management of T2DM patients and to investigate the association between their levels and insulin resistance. The study included 100 T2DM patients, which are divided into three groups according to disease duration: D1 (n=38; < 5 years), D2 (n=37; 5-10 years), D3 (n=25; >10 years), and 50 healthy persons as control (C). Fasting blood glucose (FBG) was determined by glucose oxidase-peroxidase and glycated haemoglobin A1c (HbA1c) by immunoturbidimetric assays. Serum insulin and hepcidin were evaluated using ELISA. Also, HOMA-IR and HOMA-β were calculated from FBG and insulin. The FBG, HbA1c, insulin, and HOMA-IR levels increased significantly, while HOMA-β and hepcidin levels decreased significantly in all patient groups compared to C. A significant difference in hepcidin levels was recorded between males and females in all groups. There was a significant negative correlation between HOMA-IR and hepcidin levels in all patient groups. Based on the results, it can be concluded that these biochemical markers are important in diagnosing and managing T2DM.

Keywords: Diabetes, Glycosylated hemoglobin A1c, Insulin, Insulin Resistance, HOMA-IR, HOMA- β.

العلاقة بين مستوى الهبسيدين في المصل ومقاومة الانسولين لدى المرضى العراقيين المصابين بداء السكري من النوع الثاني

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الخلاصة

هدفت الدراسة الحالية إلى تقييم تأثير مستويات الهيبسيدين في تشخيص وعلاج مرضى السكري من النوع الثاني ومعرفة العلاقة بين مستوياته ومقاومة الانسولين لدى هؤلاء المرضى. شملت الدراسة 100 مريض مصاب بداء السكري من النوع الثاني تم تقسيمهم إلى ثلاث مجموعات وفقًا لمدة الاصابة بالمرض: D1 (38 مريضا" ، أقل من 5 سنوات)، D2 (37 مريضا"، 5–10 سنوات) ،D3 (25 مريضا"، > 10 سنوات)، و 50 شخصاً سليماً كمجموعة سيطرة (C). تم قياس نسبة الكلوكوز في الدم للصائم (FBG) بطريقة الكلوكوز اوكسيديز–بيروكسيديز، و خضاب الدم السكري A1c (HbA1c) بطريقة مقياسية التعكر المناعية. تم تقييم مستويات الأنسولين و الهيبسيدين في المصل بواسطة ELISA.ايضا"، تم حساب HOMA–IR و HOMA– β من نسبة الكلوكوزفي الدم والانسولين للصائم. زادت مستويات FBG وHbA1c والأنسولين وHOMA–IR بشكل ملحوظ

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بينما انخفضت مستويات HOMA−β والهبسيدين بشكل ملحوظ في جميع مجموعات المرضى مقارنة بالمجموعة C. تم تسجيل اختلاف ملحوظ في مستويات الهيبسيدين بين الذكور والإناث في جميع المجموعات. كما كان هناك ارتباط سلبي معنوي بين مستويات HOMA−IR والهبسيدين في جميع مجموعات المرضى. بناءً على النتائج، يمكن استنتاج وتسليط الضوء على أهمية هذه المعلمات البايوكيميائية في تشخيص وعلاج مرض السكري من النوع الثاني (T2DM).

1. Introduction

Diabetes mellitus (DM) is a major public health concern that affects over 400 million people globally [1]. It is a metabolic disorder brought on by either decreased insulin action, insulin lack secretion, or both that progresses to persistent microvascular, macrovascular, and neuropathic life-threatening consequences [2,3]. Insulin, a key anabolic hormone, significantly influences the metabolism of proteins, fats, and carbohydrates [4]. Most people with DM fall into one of two groups: type 1 diabetes mellitus (T1DM), which is caused by not having enough insulin, and type 2 diabetes mellitus (T2DM), which is caused by insulin resistance (IR) and not enough insulin production to make up for it [5]. The majority of individuals have T2DM, according to the International Diabetes Federation (IDF) [6]. In Iraq, the adult diabetes prevalence is 10.4%, which means that more than three million people are diabetic [7]. Two chains of amino acids joined by disulfide bridges make up the polypeptide insulin. It is produced by pancreatic islets' rough endoplasmic reticulum. Insulin acts on adipose tissue as well as smooth, skeletal, cardiac, and hepatic muscles. The body has a wide variety of cell types with insulin receptors, including those in which insulin does not improve absorption [8]. Hemoglobin (Hb) has chemically bound to a sugar to form glycated hemoglobin (HbA1c). HbA makes up 97% of adult human Hb, followed by HbA2 (2.5%) and HbF (0.5%). HbA1c is produced when glucose condenses with each β-chain of HbA's N-terminal valine residue to form an unstable Schiff base. HbA1c, which comprises around 80% of HbA1, makes up the bulk of HbA1 [9]. In practice, HbA1c represents the average blood glucose levels throughout the previous three months, with a higher HbA1c value (recommended level in non-diabetic persons ≤ 6) [10]. Insulin resistance (IR), also known as the biological response decline of target tissues to insulin stimulation, is considered a key effector in metabolic syndrome. It impairs glucose disposal, which causes a compensatory increase in insulin production by β-cells, leading to hyperinsulinemia. T2DM is the most common IR consequence. The concentration of glucose in the blood may surpass the usual range and have serious health repercussions, depending on the dietary circumstances [11-13]. The IR and malfunction of insulin secretion can be measured using a variety of techniques. The Homeostatic Model Assessment of IR (HOMA-IR) and Homeostatic Model Assessment of β-Cell (HOMA-β) formulas are the most commonly utilized techniques in epidemiological research and are used to assess IR and β-cell function, respectively [14]. Furthermore, both IR and dysfunction of the β-cell contribute to high levels of FBG, as well as progressive deterioration from impaired glucose regulation to T2DM. While the signs of impaired glucose regulation start to appear, the FBG may still be in the normal range. When FBG levels rise in individuals with T2DM, β-cell secretory ability decreases by about 75% [15]. In 2000, researchers first discovered the hepcidin peptide in human urine. Two years later, the HAMP (hepcidin antimicrobial peptide) gene that produces hepcidin was first identified [16]. Hepcidin is an antimicrobial peptide hormone produced by the liver [17,18], which is synthesized primarily by the hepatocytes but also in macrophages and adipocytes in a small quantity [19]. Human plasma and urine contain this peptide hormone, which consists of 25 amino acids [20]. Hepatic production of hepcidin adversely regulates iron hemeostasis, leading to iron overload. By causing internalization and destruction of the iron exporter ferroprotin in these cells, hepcidin blocks iron efflux from enterocytes, macrophages, and hepatocytes into the plasma [21,22]. Hepcidin has been associated with metabolic illnesses in humans, including polycystic ovary syndrome (PCOS),

obesity, obesity-associated T2DM, and insulin resistance (IR) [23]. The vast majority of studies have focused on the role of hepcidin in T2DM and other illnesses; some studies have found higher hepcidin levels in T2DM patients, whereas others have found lower amounts [24]. Many factors, such as inflammation, morbid obesity, and chronic renal impairment, are present in patients with elevated hepcidin levels, contributing to the elevated hepcidin levels in T2DM patients. Though the cause of accompanied T2DM with low hepcidin levels remains unclear, IR is believed to play a significant role. This implies that controlling the production of insulin may be able to lower hepcidin levels [25]. According to a previous study, treating insulin levels may be a unique way to lower hepcidin levels. Moreover, it lowers the risk of diabetes and prevents cellular iron overload [26]. Therefore, evidence points to the possibility that low hepcidin levels combined with IR can lead to overt diabetes by increasing cell dysfunction via iron overload. Only recently has the role of cells in this mediation become clear [27]. Sam *et al*.'s 2013 study found a correlation between low hepcidin levels and IR. The authors used HOMA-IR as an index of IR, and their results indicated that HOMA-IR was significantly correlated with low hepcidin levels [28]. Thus, this study will be conducted to investigate the effects of T2DM duration on the serum hepcidin level, as well as the association between its levels and IR in Iraqi patients with T2DM, and the possible role of these markers in diagnosis and managing of T2DM patients.

2. Samples and Methods

2.1. Patients and control subjects

One hundred T2DM patients were included in the study; they were divided into three groups based on the duration of their disease: group D1 (38) diabetes patients (< 5 years), group D2 (37) diabetes patients (5 to 10 years), and group D3 (25) diabetes patients (> 10 years) according to standard American Diabetes Association (ADA) criteria. Fifty healthy individuals were also enrolled as the control group. Every sample was taken from patients at the Al-Kindi Teaching Hospital in Baghdad who were deemed by the medical expert to be free of issues related to diabetes. The Ethics Committee of the College of Science at the University of Baghdad approved the study protocol (Ref. CSEC/0123/0012). Table 1 presents the demographic details of the patients and control groups. Among the control participants, 50% were male and 50% were female. The representation of males and females was 58% and 42% in D1, 43% and 57% in D2, and 48% and 52% in D3.

Table 1: The demographic details of patients and control groups

Characteristics		Groups			
		C	D1	D2	D3
Number (n)		50	38	37	25
Age (year)	Mean±SD	43.14±11.05	42.79±9.89	54.29±10.47	57.24±11.24
	Min-Max	20-70	20-60	27-70	40-74
Sex	Male (%)	25 (50)	22 (58)	16 (43)	12 (48)
	Female (%)	25 (50)	16 (42)	21 (57)	13 (52)
Duration (year)	Mean±SD	-	2.00±1.12	7.92±2.24	17.36±5.66
	Min-Max	-	1.0-4.0	5.0-10.0	11.0-30.0

2.2. Exclusion criteria

The patients who have T1DM and other diabetic complications include neuropathy, retinopathy, nephropathy, cardiovascular diseases (CVD), or any other diseases that may interfere with the studied parameters. The study also eliminated people with a history of smoking or drinking alcohol.

2.3. Blood samples

Following an 8-12 hour overnight fast, blood samples (7 mL) were taken from the patients and the control group. The samples were divided into two aliquots of two and five milliliters each, which were created from the blood samples. The first aliquot was put in an EDTA-containing tube and used to estimate HbA1c, while the second aliquot was put in a gel-plain tube, let stand at room temperature for ten minutes, and then centrifuged at 3000 rpm for ten minutes in order to extract serum. The serum samples were then divided into aliquots, placed in eppendorf tubes, and kept at -20 °C until used.

2.4. Methods

Fasting blood glucose (FBG) was quantified using an enzymatic colorimetric technique using a commercially available kit (Spinreact, Spain). In summary, the oxidation of glucose to gluconic acid was catalyzed by glucose oxidase, and the formed hydrogen peroxide (H_2O_2) was detected by a chromogenic oxygen acceptor, phenol, 4-aminophoenazone (4-AP), in the presence of peroxidase. The color then corresponds to the glucose concentration in the sample. The HbA1c was measured by immunoturbidimetric assay with an automatic analyzer (Spinreact, Spin 240, Spain) for the directed kit (PZ Cromay, Poland). The fasting serum Insulin and hepcidin were measured using an ELISA kit (Human, Germany), which is based on the standard sandwich ELISA according to the manufacturer's instructions by Human Reader HS. Briefly, the standards and samples were pipetted into the well, which was coated with the antibody. Then, the primary antibody was added, followed by incubation. Secondary antibodies and chromogen substances are added. Thereafter, the color formed was read in the absorbance value at 450 nm. Insulin resistance was assessed by determining the homeostasis model estimation of insulin resistance (HOMA-IR) from levels of fasting insulin and glucose by using the following equation to compute [29]:

HOMA- IR = [Fasting blood glucose (mg/dl) × Fasting Insulin (µIU/ml)] / 405

The homeostasis assessment of β-cell function was calculated using the following equation [30]:

HOMA-β = [Fasting insulin (µIU /ml) × 360 / (Fasting blood glucose (mg/dl) - 63]

2.5. Statistical analysis

The data were analyzed using SPSS (version 22) and presented as mean ± standard deviation (±SD). In order to compare the groups, a one-way analysis of variance (ANOVA) and Kruskal-Wallis were used, as applicable. The difference between groups was defined as statistically highly significant if $p<0.01$, significant if $p<0.05$, and non-significant if the $p>0.05$. The Pearson correlation coefficient was used to determine the correlations between variables.

3. Results

Table 2 lists the glucose-related parameters, including FBG, HbA1c, insulin, HOMA-IR, and HOMA-β, as well as the hepcidin level in patients and control groups. The results in Table 2 revealed a highly significant increase ($p<0.001$) in serum FBG for all patient groups D1, D2, and D3 as compared to the C group, as well as significant variation across the D1 with D2 and D3 groups.

Table 2: Comparing biochemical variables in patients and control groups

Variables (Mean±SD)	Groups				*p*-Value
	C (n=50)	D1 (n=38)	D2 (n=37)	D3 (n=25)	
FBG (mg/dL)	92.24 ± 8.58	186.58 ± 78.34	228.73 ± 72.09	237.36 ± 89.02	0.0001 [a,b,c] 0.005 [d], 0.003 [e] 0.606 [f]
HbA1c (%)	4.39 ± 0.79	7.66 ± 2.05	7.94 ± 1.93	8.66 ± 1.32	0.0001 [a,b,c] 0.448 [d], 0.015 [e] 0.080 [f]
Insulin (µIU /mL)	2.44 ± 0.90	3.80 ± 1.96	4.78 ± 2.54	5.72 ± 2.77	0.009 [a], 0.0001 [b,c] 0.074 [d], 0.001 [e] 0.056 [f]
HOMA-IR	0.56 ± 0.21	2.11 ± 1.17	2.64 ± 1.53	2.61 ± 1.70	0.0001 [a,b,c] 0.103 [d], 0.126 [e] 0.900 [f]
HOMA-β	32.58 ± 13.17	10.38 ± 8.46	12.57 ± 10.21	18.55 ± 11.59	0.0001 [a,b,c] 0.468 [d], 0.007 [e] 0.027 [f]
Hepcidin (ng/mL)	67.96 ± 8.51	24.21 ± 4.952	24.348 ± 4.88	23.80 ± 3.86	0.0001 [a,b,c] 0.924 [d], 0.801 [e] 0.737 [f]

The *p*-value is considered a significant difference between a: (C and D1), b: (C and D2), c: (C and D3), d: (D1 and D2), e: (D1 and D3), and f: (D2 and D3).

The levels of HbA1c in groups D1, D2, and D3 were significantly higher ($p<0.001$) when compared to the control group, and D3 shows a significant increase ($p=0.015$) compared to D1. Groups D1, D2, and D3 showed a highly significant increase in insulin levels in comparison to group C. While the D3 group has a highly significant increase in insulin level compared to group D1 (p=0.001). In contrast to their level in group C, a statistically significant rise was seen in the HOMA-IR levels in all patient groups ($p<0.001$). Furthermore, the D1, D2, and D3 groups showed a significant decrease compared to the C group, and the D3 group showed a significant increase compared to D1 ($p = 0.007$) regarding HOMA-β levels. In addition, hepcidin levels decreased highly significantly for all patient groups (D1, D2, and D3) in comparison to the C group ($p<0.001$), as indicated in Table 2, while no significant differences were observed between patients in the D1, D2 and D3 groups. Furthermore, Figure 1 represents the differences between males and females regarding hepcidin levels. The results indicated that there was a statistically significant difference between males and females in all groups (C, D1, D2, and D3).

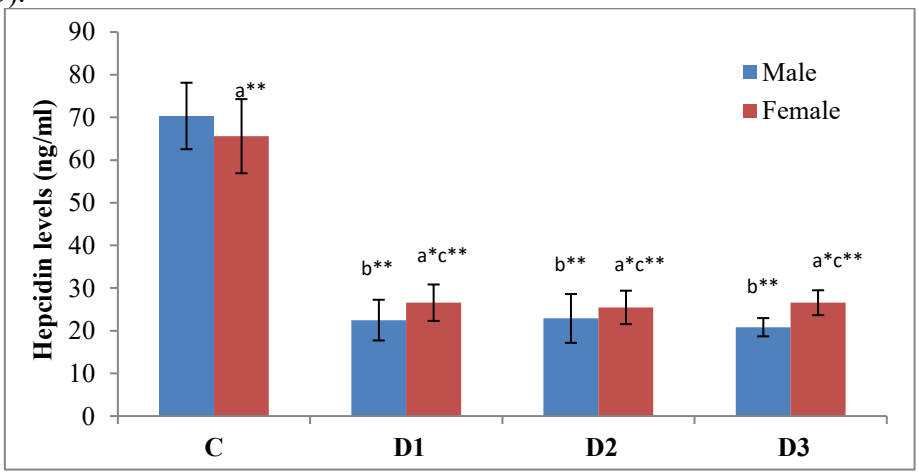

Figure 1:- Hepcidin levels in male and female patients, as well as control groups *$p < 0.05$, **$p < 0.01$. The small letters indicate a significant difference between males and females in

the same group; b indicates a significant difference between male in different groups; c indicates significant difference between female in different groups.
Tables 3, 4, and 5 illustrate the correlation among all parameters in the D1, D2, and D3 groups, respectively. In the D1 group, a highly significant negative correlation was observed between FBG and HOMA-β, whereas a highly significant positive correlation was found between FBG with HbA1c and HOMA-IR. A highly significant positive correlation was noticed between (HbA1c with insulin) and (HOMA-IR and HOMA-β with insulin). Furthermore, a significant negative correlation was found between HOMA-IR and hepcidin and HOMA-β and HbA1c.

Table 3: Pearson correlation coefficients (r) between the biochemical variables in the D1 group

Variables	HbA1c		Insulin		HOMA-IR		HOMA-β		Hepcidin	
	r	*p*	r	*p*	r	*p*	r	*p*	r	*p*
FBG	0.701**	0.0001	-0.054	0.748	0.664**	0.0001	-0.590**	0.0001	0.177	0.287
HbA1c	-	-	0.129	0.441	0.591**	0.0001	-0.363*	0.025	0.214	0.198
Insulin	-	-	-	-	0.674**	0.0001	0.618**	0.0001	0.270	0.101
HOMA-IR	-	-	-	-	-	-	0.040	0.811	-0.353*	0.030
HOMA-β	-	-	-	-	-	-	-	-	-0.060	0.719

*p< 0.05 level; ** p<0.01 level

In group D2, a moderately significant positive correlation between FBG and HbA1c, FBG and HOMA-IR, HOMA-IR and Insulin, and HOMA-β and Insulin was found. While HOMA-β and FBG showed a highly significant negative correlation. Also, a significant negative correlation was obtained between hepcidin and HOMA-IR and HOMA-β, as shown in Table 4.

Table 4: Pearson correlation coefficients (r) between the biochemical variables in the D2 group

Variables	HbA1c		Insulin		HOMA-IR		HOMA-β		Hepcidin	
	r	*p*	r	*p*	r	*p*	r	*p*	r	*p*
FBG	0.490**	0.002	-0.199	0.237	0.452**	0.005	-0.617**	0.0001	0.281	0.092
HbA1c	-	-	0.001	0.996	0.324	0.050	-0.172	0.309	0.166	0.325
Insulin	-	-	-	-	0.743**	0.0001	0.763**	0.0001	-0.292	0.080
HOMA-IR	-	-	-	-	-	-	0.216	0.200	-0.359*	0.047
HOMA-β	-	-	-	-	-	-	-	-	-0.375*	0.022

*p< 0.05 level; ** p<0.01 level

In the D3 group, Table 5 shows that insulin has a strong positive relationship with both HOMA-IR and HOMA-β. It also shows that FBG and HbA1c, HOMA-IR and FBG, and HOMA-IR and HbA1c all have a significant positive relationship. There was a highly negative correlation between FBG and HOMA-β while there was a significant negative correlation between hepcidin, insulin and HOMA-IR.

Table 5: Pearson correlation coefficients between the biochemical variables in the D3 group

Variables	HbA1c		Insulin		HOMA-IR		HOMA- β		Hepcidin	
	r	*p*	r	*p*	r	*p*	r	*p*	r	*p*
FBG	0.318*	0.035	-0.276	0.181	0.466*	0.019	-0.762**	0.0001	0.152	0.467
HbA1c	-	-	0.273	0.186	0.411*	0.041	0.130	0.535	-0.040	0.849
Insulin	-	-	-	-	0.659**	0.0001	0.718**	0.0001	-0.454*	0.023
HOMA-IR	-	-	-	-	-	-	0.020	0.924	-0.310*	0.031
HOMA-β	-	-	-	-	-	-	-	-	-0.233	0.262

*p< 0.05 level ; ** p<0.01 level

4. Discussion

Type 2 diabetes mellitus (T2DM) is a metabolic disease marked by IR and pancreatic β-cell damage as a result of unstable hyperglycemia [31]. In 2017, the IDF reported that 38.7 million people in the Middle East and North Africa (MENA) area were living with diabetes, with over half of these cases remaining undiagnosed [32]. Additionally, estimates suggest that about 73 million adults in the age range of 20 to 79 years will have diabetes by 2021. Additionally, experts predict that the number of diabetics in the MENA region will rise at the second-highest rate by 86%, reaching 136 million by 2045. According to reports, the prevalence of DM has increased dramatically in Iraq over the past years, from 19.58/1000 in 2000 to 42.27/1000 in 2015. This increase is consistent with global trends for the disease [33]. Therefore, considering its high prevalence rate, rising incidence rate, and overall financial burden, diabetes is a major public health concern for Iraqis. The current study reports a higher FBG level among T2DM patients, a finding that aligns with previous research [34]. Diabetes duration is one of the most powerful predictors of complication risk, including cardiovascular and kidney diseases. Longer-term sick individuals also had higher age and FBG levels; this suggests that the prevalence of severe hyperglycemia increased with both duration and age [35]. The level of HbA1c in the blood depends on both the red blood cell lifetime and glucose concentration [36]. As a result, the current research indicates a large increase in the HbA1c level of T2DM patients compared to healthy controls, with a high level in the longer-term duration D3 group. This result is consistent with earlier research, which discovered that the duration of diabetes increased and the HbA1c value increased in both the elderly and non-elderly groups [37]. Also, the increased concentration of FBG in the patients in the current study is associated with an increase in HbA1c levels in these individuals (D1 and D2 groups). The study by Ikekpeazu *et al*. [38], which reported a positive association between FBG and HbA1c, is consistent with the results. The American Diabetes Association (ADA) recommended a diagnosis of diabetes with an HbA1c of 6.5 and the highest risk of diabetes development with a HbA1c of 5.7-6.4 [39]. In terms of insulin levels, similar findings were reported by Alshawk *et al*., who revealed that insulin levels were higher in T2DM patients compared to control people [40]. According to Vladu *et al*., patients with diabetes had a larger percentage of IR when compared to people without diabetes [41]. The findings in Table 2 record insulin, HOMA-IR, and HOMA-β, which is consistent with previous research by Hussein and Saifalla, who found that insulin sensitivity was negatively associated with fasting insulin and that insulin levels rose with diabetes duration [42]. The reality that the body becomes more resistant to insulin as the disease's duration increases, along with the impact of medications that may reduce cell resistance to insulin, could explain the current data [43]. It is extremely exciting to evaluate insulin resistance/sensitivity and pancreatic β-cell activity in order to diagnose the type of diabetes and predict a successful course of therapy and prevention. Researchers have developed a variety of methods and indices to assess insulin sensitivity, resistance, and cell function to date. These methods include computations and variable models like dynamic and static tests [42,44]. Because insulin testing

is used to help diagnose early T2DM, the current finding makes sense; on the other hand, these results might be the outcome of β-cell malfunction. According to a previous study, β-cell function levels in T2DM patients were considerably lower than the control group [45]. In normal physiology, increased plasma glucose levels stimulate insulin production. Patients with T2DM have shown greater amounts of IR, which they link to increased insulin levels. These findings are in accordance with a study [46] that found a strong and significant correlation between insulin level and HOMA-IR. The fact that IR is most likely the earliest metabolic aberration in T2DM, leading to elevated pancreatic hypersecretion of insulin, explains this discovery. When hyperglycemia is chronic and protracted, the pancreatic β-cells become damaged and cease to function [47]. Understanding hepcidin's role is critical because excess iron increases the risk of diabetes. Hepcidin expression has been described in both pancreatic β-cells and adipose tissue in the past few years [48,49]. Despite its extremely low concentrations, this suggests that hepcidin may contribute to cardiometabolic risk through mechanisms beyond its role in limiting iron absorption. Anemia, hypoxia, and iron deficiency all reduce hepatic production of hepcidin, but hepcidin remains throughout an acute phase response [17]. Furthermore, hepcidin has been associated with diseases such as iron overload, PCOS, gestational diabetes mellitus (GDM), and hereditary hemochromatosis [50-52]. In the present study, new findings revealed that about three durations of T2DM were associated with decreased levels of hepcidin. Guo *et al*.'s research found no statistically significant association between hepcidin concentrations and glycemia in either the non-diabetic or diabetic groups [53], which aligns with the recent findings. According to the results of this research, a low hepcidin level has been revealed in T2DM, which contradicts the findings of Al-Adhami *et al*. and Jiang *et al*. [54,55]. Al-Adhami *et al*., who have described that higher hepcidin concentrations in T2DM may be attributed to higher ferritin concentrations, raised hepcidin adaptive value by down-regulating iron absorption, and play a crucial role in the development of T2DM [54]. According to Jiang *et al*., people with T2DM had considerably greater hepcidin concentration than age-matched controls, but they did not adjust for body mass index (BMI), which was significantly higher in the diabetic group [55]. However, our study explicitly eliminated confounding factors like inflammatory processes and smoking. There is evidence that higher levels of oxidative stress lead to more iron-related protein synthesis. This means that smoking may raise levels of oxidative stress and indirectly change iron status markers like ferritin [56]. The combination of smoking and other substances causes increased iron release from alveolar macrophages [57]. Furthermore, smoking can trigger subclinical inflammatory responses due to its pro-altherogenic action [58] and potentially confuse cardiometabolic results. Therefore, by excluding individuals who have inflammatory processes and smoke, the bias of enrolling unhealthy control and individuals with co-morbidity is greatly reduced. On the other hand, Vela *et al*. and Suárez *et al*. have observed that circulating hepcidin levels were significantly reduced in people with T2DM [27,59]. According to previous research, there are two types of hepcidin disruption in T2DM: the first has high levels and the second has low levels of hepcidin. The first high level is associated with people who have upregulators of hepcidin expression (chronic renal failure, morbid obesity, high degree of inflammation) [60], and the second low level is associated with IR [27]. IR is the primary pathological characteristic of T2DM. The current result showed an inverse correlation between the hepcidin level and HOMA-IR, aligning with the findings reported by Zargham *et al*. [61]. Furthermore, several studies have presented the link between IR and hepcidin levels. A 2013 study found an inverse relationship between low levels of hepcidin and IR. The authors of that study employed homeostatic model assessment of IR (HOMA-IR) as an indication of IR, and their findings revealed that HOMA-IR was substantially connected with low levels of hepcidin [28]. Another study also found that people with IR and T2DM had less hepcidin messenger ribonucleic acid (mRNA) in their livers [62]. In comparison to the control group, Le Guenno *et al*. observed a 3.5-fold decrease in hepcidin mRNA in the group with higher IR [63]. In a different study,

people with T2DM had lower levels of pro-hepcidin and hepcidin, which suggests that they lose the insulin signal when their iron stores rise [28]. It has been discovered that decreased hepatic insulin signaling caused by IR reduces hepcidin synthesis via downregulating signal transducer and activator of transcription 3 (STAT3)-mediated pathways [26,27]. Moreover, other mechanisms can impact the levels of hepcidin in T2DM patients. Hepcidin is known to be most highly expressed in beta cells in pancreatic tissue. Also, beta cells co-secrete insulin and hepcidin, reinforcing the connections between iron and glucose metabolism [64]. Interestingly, research done by Vela and colleagues [26] revealed that hepcidin levels in T2DM patients receiving insulin therapy remain unchanged, potentially as a consequence of insulin therapy. In this situation, insulin therapy represents a cutting-edge approach to hepcidin level adjustment. Thus, by adjusting hepcidin levels, we can avoid cellular iron overload and lower the risk of diabetes. The current study found that T2DM patients had lower levels of hepcidin, a hormone involved in iron metabolism. This is consistent with previous research showing an association between low hepcidin levels and IR. Therefore, low hepcidin levels are a result of IR; however, insulin treatment that corrects insulin signaling may bring these levels back to normal in T2DM patients. To provide a more thorough understanding of the relationship between hepcidin levels and IR, these pathways should be investigated further to develop effective strategies for T2DM treatment.

5. Conclusion

The study on T2DM in Iraq found that these patients had higher FBG and HbA1c levels compared to healthy individuals. This indicates poor blood glucose control in diabetic patients. Insulin levels were also higher, suggesting IR. Also, the glycemic control parameters increased with disease duration. All disease durations showed low levels of hepcidin, a hormone involved in iron metabolism, in T2DM patients, which is associated with IR. These findings highlight the importance of these biochemical markers in diagnosing and managing T2DM.

Ethical Clearance

The Research Ethical Committees of the College of Sciences/University of Baghdad accepted this study protocol (Ref. CSEC/0123/0012).

Conflict Clearance

According to the authors, there is no conflict of interest.

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