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Research Article

THE RELATIONSHIP BETWEEN SERUM FERRITIN AND INSULIN RESISTANCE IN TYPE 2 DIABETIC PATIENTS TREATED BY METFORMIN

Mahmood A. Al-Hafidh Khattab[1] and Wahda Basheer Al-Youzbaki[2*]

[1]Ministry of Health, Nineveh Health Directorate, Mosul – Iraq.
[2]Department of Pharmacology, College of Medicine - University of Mosul – Iraq.

ABSTRACT

Background: It has been hypothesized that serum ferritin could be a marker of insulin resistance and that serum ferritin may also be an independent determinant of poor metabolic control in the diabetic patient. **Objective:** To evaluate the effect of metformin on the relationship between serum ferritin and glycemic control and insulin resistance in type 2 diabetes mellitus (T2DM) patients. **Methodology:** This is a case control study conducted from the period of the 15th of April 2013 to the 31st of December 2013 on 100 patients with T2DM, their age range was between (40-70) years. They were divided into two groups, the 1st group consisted of fifty T2DM (24 males and 26 females), , treated by metformin tablets only, in doses ranged from 500-1700 mg/day. Group 2 consisted of fifty newly diagnosed T2DM on diet for 1-3 months (22 males and 28 females). Forty nine apparently healthy subjects (29 males and 20 females), also their age range was between (40-70) years, were included in this study as a control group. Part of the whole blood sample which was obtained after overnight fasting from the three groups was used to measure HbA1C and the sera from the remaining blood samples were used to measure fasting serum glucose (FSG), serum ferritin and insulin by commercial kits while HOMA-IR was calculated by special equation. **Results**: This study demonstrated a significant higher level of the mean serum ferritin and Hb in the male diabetic group on diet and the male diabetic group on metformin in comparison with the male healthy control group, but a non significant differences in the mean serum ferritin and Hb level in the two male diabetic groups. There were a significant higher mean serum ferritin level but a non significant difference in the mean Hb level of the female diabetic on diet group and the female diabetic group on metformin in comparison with female healthy control group, but a non significant differences in the mean serum ferritin and Hb level in the two female diabetic groups. This study showed a significant higher level of the mean FSG and HbA1c level in the diabetic on diet group and the diabetic on metformin group in comparison with healthy control group, but a significant lower level of the mean FSG and a non-significant lower level of HbA1c in the diabetic on metformin group in comparison with diabetic on diet group. There were a significant lower level of mean serum insulin and HOMA-IR value in the diabetic on metformin group in comparison with diabetic on diet group. **Conclusion:** There were non-significant differences between mean serum ferritin level in diabetic on metformin in comparison with diabetic on diet in male and female patients. Metformin for more than six months caused a significant glycemic control, and a significant reduction in insulin resistance in comparison with diabetic on diet groups, but there were no significant correlation between mean serum ferritin level and glycemic control, nor with insulin resistance parameters.

Keywords: diabetes mellitus, ferritin, insulin, insulin resistance.

INTRODUCTION

The role of micronutrients in the etiology of T2DM is not well established[1]. Fernández-Real *et al.,* (1998)[2] hypothesized that serum ferritin could be a marker of insulin resistance and concluded that serum ferritin may also be an independent determinant of poor metabolic control in the diabetic patient.

Several epidemiological studies have reported a positive association between high body iron stores, as measured by circulating ferritin level, and the risk of T2DM[2-4]**.** Although a mechanism linking iron concentrations and diabetes is yet to be established. In fact, it has been demonstrated that increased iron stores may contribute to insulin resistance by reducing hepatic insulin extraction and metabolism[5], and by decreasing glucose uptake in muscle, so decreasing insulin sensitivity[5,6]. In addition, increased dietary intake of iron, especially that of heme iron, is associated with risk of T2DM in apparently healthy populations[7]. Furthermore, a clinical trial[6] suggested that phlebotomy induced reduction in body iron levels may improve insulin sensitivity in humans.

Level of serum ferritin, a predominant iron-storage protein and a biomarker of iron stores**,** are elevated in persons with prevalent diabetes as compared with non diabetic controls [8] and correlate with impaired fasting glucose levels[9]. In addition, several cross-sectional**,** case-control studies and prospective studies have identified an independent association between baseline elevations in iron stores and the occurrence of T2DM, in different association in men and women[3,4,9-12]. In epidemiological study, involving 1,013 men[13], serum ferritin was the second-strongest determinant of blood glucose (after BMI) in regression models and the third-strongest determinant of serum insulin (after BMI and age). However, several questions remain unanswered.

Metformin as insulin sensitizer, its blood glucose-lowering actions result primarily from an amelioration of insulin resistance, mainly in liver and muscle, with a lesser effect in adipose tissue[14]. Luque-Ram´irez *et al.,* (2007)[15] concluded that increased body iron stores of obese women with PCOS are a consequence of insulin resistance and hyperinsulinism and are not a result of reduced menstrual losses by finding that despite the fact that treatment with Diane35 Diario restored regular menstrual cycles in all the patients, whereas metformin only did so in 50% of them, serum ferritin levels decreased at 12 and 24 weeks of treatment only with metformin, in association with a marked increase in insulin sensitivity. On the contrary, no changes in ferritin and insulin sensitivity were observed with Diane35 Diario.

However, most of the epidemiological studies[2,4,7,16-19] that have reported strong association between serum ferritin and increased risk for T2DM appear not to have taken the aetiological effect of the therapeutic regimen into serious consideration when interpreting the observed relationship. Hence, the need to ascertain what effect therapeutic regimen especially metformin as insulin sensitizer drug has on the relationship between serum ferritin and glycemic control and insulin resistance T2DM patients which is the goal of this study.

Methodology

This is a case control study conducted on 100 patients with T2DM who were registered at Al-Wafaa Center of Diabetes Management and Research in Mosul from the period of the 15th of April 2013 to the 31st of December 2013. They were divided into two groups, the 1st group consisted of fifty T2DM (24 males and 26 females), treated by metformin tablets monotherapy for at least 6 months, in doses ranged from 500-1700 mg/day. Group 2 consisted of fifty newly diagnosed T2DM (22 males and 28 females), on diet for 1-3 months. Those diabetic patients, age range between 40-70 years, have no other concomitant diseases like hypertension, thyroid disease, renal and hepatic diseases nor using other concomitant drugs, were included in this study. Patients with T1DM**,** pregnant and lactating women, smokers , alcohol drinking patients were excluded from the study. Forty nine apparently healthy subjects (29 males and 20 females), their age range was also between (40-70) years were included in this study as a control group.

Part of the whole blood sample which was obtained after overnight fasting from the three groups was used to measure HbA1C and the sera from the remaining blood samples were used to measure:

1-Serum ferritin using chemiluminescent immunoassays technique (CLIA), using Insulin LIAISON kit supplied by Diasorin, Saluggia (VC)-Italy, and calculated by LIAISON immunoanalyzer.

2- Fasting serum glucose (FSG) using a kit supplied by Biocon Company (Germany),

3- Serum insulin using chemiluminescent immunoassays technique (CLIA), using Insulin LIAISON kit supplied by Diasorin, Saluggia (VC)-Italy, . and calculated by LIAISON immunoanalyzer.

HOMA-IR was calculated by the following equation:

HOMA-IR= fasting serum insulin (µIU/ml) × fasting plasma glucose (mg/dl)/405 [20] .

Patients were considered as insulin resistant when HOMA ≥ 2.6 [21].

Statistical Analysis

The data obtained in the current study was analyzed using statistical package for social sciences (SPSS) **(version 12).** Standard statistical methods were used to determine the mean and standard error. Unpaired t-test was used to compare between healthy and diabetic studied groups. One way ANOVA and Post Hoc (Duncan) test were used to identify statistical difference through comparison within and among groups.

The approval of the study protocol by an ethic committee has been obtained from the local health committee of College of Medicine – University of Mosul – Iraq.

RESULTS

A total number of 149 subjects enrolled in this study. They were divided into 3 groups. The first group included 50 diabetic patients on metformin therapy on a dose range between (500mg- 1700mg) with mean duration of treatment was 6.93 ± 4.5 years. The second group included 50 newly diagnosed diabetic patients (on diet) for 1-3 months. The third group included 49 healthy subjects served as control.

Table (1) shows the general characteristics of the diabetic on metformin, diabetic on diet, and the healthy control group. There was no significant difference in the mean age non in the gender among the three studied groups.

Table 1: General characteristics of the diabetic patients (on metformin and on diet) and healthy control groups

Variables	Diabetics on metformin n=50	Diabetics on diet n=50	Healthy Control n=49	P-value
Age (year), mean ± SD	50.56±7.45	51.50±7.43	52.29±8.29	NS
Gender				
Males, No. (%)	24 (48.0%)	22 (44.0%)	29 (59.2%)	NS
Females, No. (%)	26 (52.0%)	28 (56.0%)	20 (40.8%)	

Table (2) demonstrates a significant higher level of the mean serum ferritin and Hb in the male diabetic on diet group and diabetic on metformin group in comparison to male healthy control group separately. Although there was a reduction in the mean serum ferritin and Hb level in the male diabetic on metformin group in comparison to male diabetic on diet group but it was not significant.

Table 2: Comparison between the mean serum ferritin and mean Hb level of male diabetic on metformin group male, diabetic on diet group and male healthy control group

Parameters	Mean± SD		
	Male diabetics on metformin (n= 24)	Male diabetics on diet (n= 22)	Male healthy control (n= 29)
S. Ferritin (ng/ml)	80.19 ± 45.02 a	87.59 ± 62.80 a	61.05 ± 8.32 b*
Hb (g/dl)	14.67 ± 45.02 a	15.16 ± 1.57 a	13.75 ± 0.05 b**

Different letters horizontally mean significant difference.
* P-value = 0.02
** P-value <0.0001

Table (3) demonstrates a significant higher mean serum ferritin level but a non significant difference in the mean Hb level of the female diabetic on diet group and female diabetic on metformin group in comparison with female healthy control group separately. Table (3) also demonstrates a non significant difference in the mean serum level of Hb and ferritin in diabetic female on metformin group in comparison to diabetic female on diet group.

Table 3: Comparison between the mean serum ferritin and mean Hb level of female diabetic on metformin, female diabetic on diet and female healthy control groups

Parameters	Mean± SD		
	Female diabetics on metformin (n= 26)	Female diabetics on diet (n= 28)	Female healthy control (n= 20)
S.Ferritin(ng/ml)	76.58 ± 46.16 a	93.38 ± 69.86 a	33.17±4.15 b*
Hb (g/dl)	13.31 ± 1.80 a	13.36 ± 0.89 a	13.01±0.65a

Different letters horizontally mean significant difference.
* P-value <0.0001

Table (4), demonstrates a significant lower level of mean serum ferritin in the female in comparison with male subjects in the healthy group, while there were a non-significant differences of the mean serum ferritin level in the female in comparison with male between diabetic on diet group and diabetic on metformin group.

Table 4: Comparison between mean serum ferritin according to gender in diabetic on metformin group, diabetic on diet group and healthy control group

gender / Group	Mean ± SD		p- value *
	Male	Female	
Healthy control group	61.05±8.32	33.17±4.15	<0.0001
Diabetic on diet group	87.59±62.80	93.38±69.86	0.7
Diabetic on metformin group	80.19±45.02	76.58±46.16	0.7

Table (5) illustrates a significant higher level of the mean FSG and HbA1c level in the diabetic on diet group and diabetic on metformin group in comparison with healthy control group separately. Table (4) also illustrates a significant lower level of the mean FSG but a non-significant lower level of HbA1c in the diabetic on metformin group in comparison with diabetic on diet group.

Table 5: Comparison between the mean FSG and HbA1c level of diabetic on metformin, diabetic on diet and healthy control groups

Parameters	Mean± SD		
	Diabetics on metformin (n= 50)	Diabetics on diet (n= 50)	Healthy control (n= 49)
FSG (mg/dl)	179.84 ± 80.47 a	210.98 ± 59.16 b*	104.02±8.36 c**
HbA1c %	7.54 ±1.66 a	8.06 ± 2.14 a	5.11±0.19 **c****

Different letters horizontally mean significant difference.

* P-value= 0.03

** P-value <0.0001

Table (6) demonstrates a significant lower level of mean serum insulin and HOMA-IR value in the diabetic on metformin group in comparison with diabetic on diet group.

Table 6: Comparison between mean serum insulin and HOMA-IR value of diabetic on metformin and diabetic on diet groups

Parameters	Mean± SD		P-Value
	Diabetics on metformin n= 50	Diabetics on diet n= 50	
Serum insulin (µ IØ ml)	18.97 ± 11.38	28.04 ± 20.86	0.009
HOMA-IR	8.40 ± 6.37	15.34 ± 16.41	0.007

Fig. (1) shows a non-significant correlation (r=0.17, p=0.2) between mean ferritin and mean FSG in the diabetic patients on metformin.

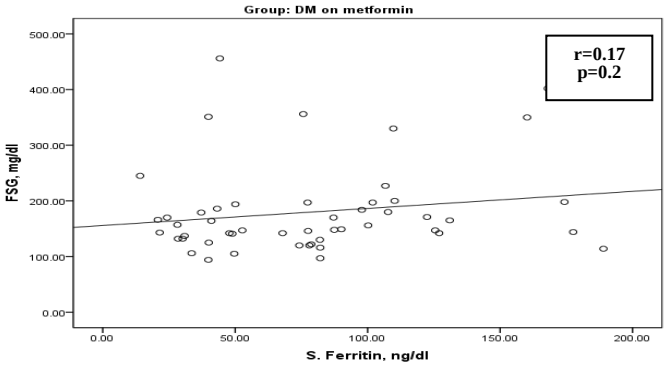

Fig. 1: Correlation between mean serum ferritin and mean serum FSG in the diabetic patients on metformin

Fig. (2) shows a non-significant correlation (r=0.09, p=0.4) between mean serum ferritin and mean HbA1c value in the diabetic patients on metformin.

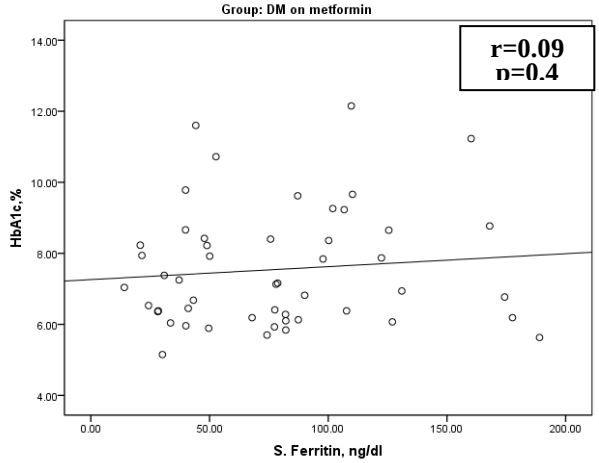

Fig. 2: Correlation between mean serum ferritin and mean HbA1c value in the diabetic patients on metformin

Fig. (3) shows a non-significant correlation (r= -0.05, p=0.6) between mean serum ferritin and mean serum insulin level in the diabetic patients on metformin.

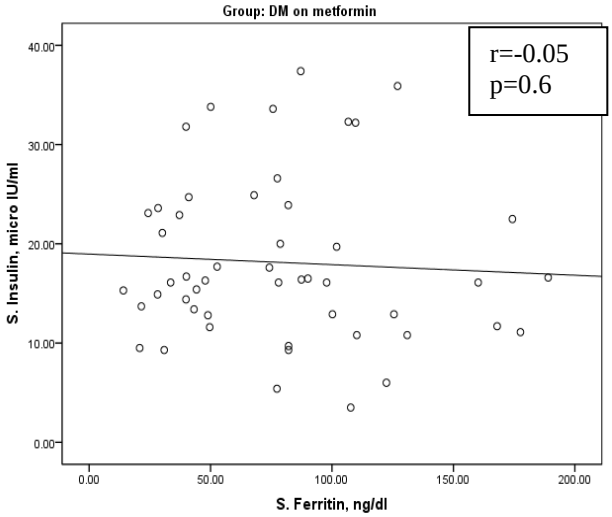

Fig. (3): Correlation between mean serum ferritin and mean serum insulin level in the diabetic patients on metformin

Fig. (4) shows a non-significant correlation (r=0.03, p=0.8) between mean serum ferritin and mean HOMA-IR value in the diabetic patients on metformin.

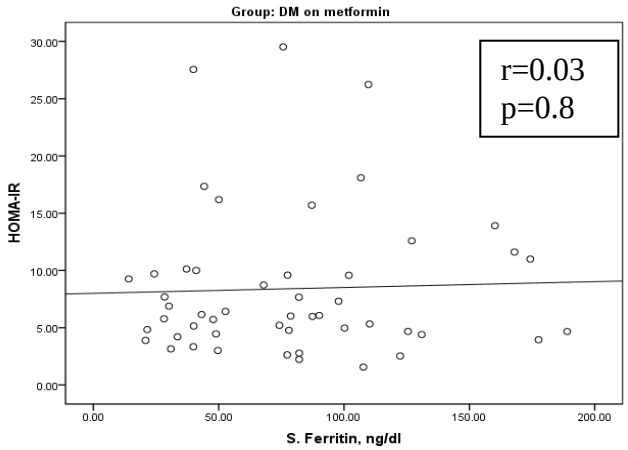

Fig. (4) : Correlation between mean serum ferritin and mean HOMA-IR value in the diabetic patients on metformin

DISCUSSION

This study showed that mean serum ferritin level is significantly higher in the diabetic group on diet (both male and female) in comparison with the healthy control group, this is in agreement with[4,22-24].

Differences in iron status exist according to sex[9,25]. This study showed that the mean serum ferritin concentration in the healthy control group was significantly higher in male compared with female subjects. This is in consistent with other studies[8,25-26]. Also this study showed that mean serum ferritin in diabetic on diet group was non significantly higher in female than in male (table 4). This is in agreement with (Louise *et al.,* 2013) [26] in that serum ferritin was more strongly associated with diabetes in women compared with men, suggesting that hormonal differences may play a role in the association between ferritin levels and T2DM risk[9,27]. Body composition is suggested to affect the association between serum ferritin and the insulin resistance syndrome, and that sex differences in serum ferritin might exist due to sex hormone levels and differences in iron accumulation in the peripheral muscles, which may cause derangement of muscle glucose uptake because of muscle damage [28].

The result of this study illustrated a significant higher level of the mean FSG and HbA1c value in both diabetic groups, (on diet group and on metformin) in comparison with healthy control group (table 5), this mean a poor glycemic control in T2DM patients treated by monotherapy with metformin which is in agreement with Derosa *et al.,* (2012)[29].

The mean FSG level and the mean HbA1c value were positively correlated with the mean serum ferritin (although non significantly) in diabetic patients on metformin group. This is in agreement with other studies[2,9,10,13,17,22-24,30].

Both blood glucose and serum insulin concentrations were only elevated at mildly increased serum ferritin concentrations[13]. The pathophysiological mechanisms underlying the unfavorable association of increased serum ferritin with IR remain unknown, although several explanations have been suggested. Iron may cause insulin action to deteriorate and impede glucose utilization in adipocytes. Accumulating iron in the liver may interfere with hepatic extraction of insulin, which can result in impaired glucose tolerance[31].

Glycemic control was correlated with elevated iron store[3]. Glycemic control itself influences serum ferritin concentrations, (glycosylated ferritin has a longer serum half-life)[30,32]. Poorly controlled patients of diabetes had hyperferritinemia, and so, serum ferritin was increased in diabetes as long as glycemic control was not achieved[33]. This is in agreement with the results of this study that found a significant higher mean serum ferritin level in both diabetic groups (diet and metformin) in comparison with the healthy control groups due to poor glycemic control represented with a significant higher level of FSG and HbA1c % value of both diabetic groups (diet and metformin) in comparison with the healthy groups (table 5). In spite of that there was a significant lower mean FSG with a non-significant lower mean HbA1c% value of diabetic on metformin group in comparison with diabetic on diet group (table 5).

The results of this study showed a significant lower level of the mean FSI and HOMA- IR in diabetic on metformin group in comparison to diabetic on diet group (table 6). These results are in agreement with other studies[34-40] who found that metformin improve insulin sensitivity in diabetic patients and even in obese and insulin-resistant individuals without diabetes[36].

This study found a non-significant negative correlation between mean serum ferritin and insulin level in diabetic on metformin group (fig.3). According to the author knowledge, no such studies on the relation between mean serum ferritin and FSI in T2DM treated by metformin was done, although there are many studies [2,9,11,22.28,31,41-44] found a significant correlation between mean serum ferritin and markers of insulin resistance in T2DM, whereas. Zafar *et al*., (2011) [45] did not found such correlation.

The initial glucose abnormalities include insulin resistance and hyperinsulinemia. Iron overload may induce insulin resistance by reducing insulin extraction in the liver[5,46-47] . Iron can disrupt insulin inhibition of hepatic glucose production, which together with reduced hepatic extraction of insulin leads to peripheral hyperinsulinaemia[5,48], followed by reducing insulin synthesis and excretion by the pancreatic beta cells which may be a result of iron deposition in these cells[46,49,]. Conversely, insulin stimulates cellular iron uptake through increased transferring receptor externalization[50]. Thus, insulin and iron can mutually potentiate their effects, leading, after a vicious cycle, to insulin resistance and diabetes. This explains the negative correlation between ferritin and FSI that found in this study in the diabetic group on metformin (Fig. 3).

Many studies have linked increased serum ferritin concentrations in non pathologic conditions, reflecting subclinical iron overload, to insulin resistance[2,41,51-52] and an increased risk of T2DM[10,53]. This is in agreement with this study that found a positive correlation (although non significant) between mean serum ferritin and HOMA-IR (fig.4).

CONCLUSION

There were non-significant differences between mean serum ferritin level in diabetic on metformin in comparison with diabetic on diet in male and female patients. Metformin for more than six months caused a significant glycemic control (although there were a significant lower level of the mean FSG but a non-significant lower level of HbA1c). Also caused a significant reduction in insulin resistance in comparison with diabetic on diet groups, but there were no significant correlation between mean serum ferritin level and glycemic control, nor with insulin resistance parameters (FSI and HOMA-IR).

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