Serum ferritin and iron in diabetic and non-diabetic with acute myocardial infarction

Hamid Sharif Nia[1], Ali Akbar Haghdoost[2], Yiong Huak Chan[3], Fariba Tabari[4], Abolfazl Hashemi[5], Babak Alaei[6], Mohammad Taghi Salehi Omran[7], Abolghasem Siyadat Panah[8*], Mohammad Ali Soleimani[9]

1- Candidate of PhD, Faculty of Nursing and Midwifery of Amol, Mazandaran University of Medical Sciences, Sari, Iran. PhD Student of Nursing at Baqiyatallah University of Medical Sciences, Tehran, Iran.
2- PhD of Epidemiology, Professor of Research Center for Modeling in Health, Kerman University of Medical Sciences, Kerman, Iran.
3- PhD of Mathematics, Head, Biostatistics Unit, Yong Loo Lin School of Medicine, National University Health System, Singapore
4- Candidate of PhD, Faculty of Nursing and Midwifery of Tehran, Tehran University of Medical Sciences, Tehran, Iran. PhD Student of Nursing at Baqiyatallah University of Medical Sciences, Tehran, Iran.
5- MD. Laboratory Sciences Babol University of Medical Sciences, Babol, Iran.
6- BSc. Faculty of Paramedical of Amol, Laboratory Sciences Mazandaran University of Medical Sciences, Sari, Iran.
7- MD, Department of cardiology and echocardiography, Babol University of Medical Sciences, Babol, Iran
8- MSc. Faculty of Paramedical of Amol, Laboratory Sciences, Mazandaran University of Medical Sciences, Sari, Iran.
9- Qazvin University of Medical Sciences, Qazvin, Iran and PhD Student of Nursing at Tehran University of Medical Sciences, Tehran, Iran

*Corresponding author: Abolghasem Siyadat Panah Email: asiyadatpanah@yahoo.com
Tel: +981212221919 Fax: +98(0121) 2151220

Abstract: An increased serum ferritin level is presented as a risk factor for coronary artery disease. The role of diabetes mellitus on serum ferritin levels in myocardial infarction has recently been investigated. the present study has been carried out to investigate Comparison of the level of ferritin and iron in type 2 diabetic and nondiabetic patients with acute myocardial infarction(AMI).This case-control study has been conducted on 200 type 2 diabetic and non-diabetic patients with AMI. Blood samples were prepared and analyzed for the comparison of serum ferritin and iron, TIBC, CBC and blood lipids between the two groups. Female gender (OR=6.68) and smoking (OR=4.03) has been found to be significantly related to diabetes (OR=4.03). The mean ferritin level was 171.20 (SD=114.35) ng/dl in the case and 168.80 (SD=124.36) ng/dl in the control group (p=0.8), and the mean iron level was 87.71 (SD=35.58) mg/dl in the case and 62.70 (SD=22.27) mg/dl in the control group (p<0.0001, OR=1.86). Blood iron and ferritin levels were lower than the standard levels in both genders of patients with myocardial infarction (p<0.0001). Regarding the results of the present study, high blood ferritin level does not bring about type 2 diabetes, and the hypothesis of ferritin level association with type 2 diabetes and its elevated level in patients with AMI has not been confirmed; high levels of iron, on the other hand, may underlie development of type 2 diabetes; therefore, further investigation seems to be necessary in this regard.

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Introduction

Diabetes mellitus is a chronic disorder with high coronary artery disease morbidity and mortality.[1, 2] Approximately 150 million worldwide are suffering from this disorder and the number is expected to increase to 300 million by 2025.[3] Patients with both diabetes mellitus and cardiovascular disease have an especially poor prognosis.[4]

former assessments have uniformly pointed remark to the increased early and late mortality after acute myocardial infarction in type 2 diabetic patients.[5] In spite of the fact that type 2 diabetes is more common than type 1 diabetes, less is known about its pathogenesis.[6] Increasing of level of glucose is relative with insulin deficiency, associated with enhanced lipolysis and elevated circulating free fatty

acids, which may damage cardiac cells.[7] Some studies have revealed that increase in blood ferritin level, as an important and independent biofactor, may lead to increased prevalence of diabetes.[8, 9, 10] Moreover, other studies have shown significant relationship between elevated blood iron levels and the incidence of coronary artery disease and myocardial infarction.[11] Ferritin is a protein-iron-phosphorus compound which is considered as an indicator of body iron stores. Iron is responsible for oxygen delivery to tissues and plays a main role in cellular oxidation. High level of serum iron contributes to increase in blood ferritin levels.[12]

Ferritin is the major iron storage protein which exists in cells of the liver, spleen and bone marrow and, to lesser extent, in heart, pancreas and kidney.[13] Although iron is essential for metabolic processes, a hypothesis has been raised on the relation between blood iron level and vessel dysfunction in type 2 diabetes.[14] As a powerful peroxidant, iron can attack cell membrane lipids and proteins and nuclear nucleic acids. It is hypothesized that iron leads to insulin resistance and consequently development of type 2 diabetes through reducing the insulin secretion, [15] and may also eventuate in coronary artery disease (CAD) by enhancing the cholesterol levels.[16]

Positive correlation has been reported by many studies between plasma ferritin level and type 2 diabetes; yet, no serious research has approved the hypothesis on increased concentration of stored iron and development of type 2 diabetes.[17] Although higher plasma ferritin level in diabetic patients compared to non-diabetic subjects has been elucidated in a cohort study by Jehn et al. (2007), no significant relationship has been found between ferritin level and diabetes as a predictable factor in causing type 2 diabetes after adjusting variables such as age, ethnicity, gender, menopausal status, smoking and BMI.[18] Similarly, findings of a study by Sharifi et al. (2004), on 97 patients with type 2 diabetes, no significant correlation has been observed between the serum ferritin and glycosylated hemoglobin and fasting blood glucose among the study samples.[11]

Albeit various studies have confirmed the relationship between high serum ferritin level and AMI,[13, 19, 20] the correlation between serum ferritin levels and the extent of CAD is still not well understood as stated by Samimi et al. (2008).[21]

With increasing prevalence of type 2 diabetes and its impact on cardiovascular disease, the link between ferritin and diabetes may be different between patients with myocardial infarction and healthy subjects, and, in other words, an interaction may exist between MI and level of ferritin on development of diabetes. As a result, this study has been merely confined to patients with myocardial infarction since such a relationship has been more addressed in healthy individuals; in this line, the present study has been undertaken to Comparison of the level of ferritin in type 2 diabetic and nondiabetic patients with acute myocardial infarction

Material and Methods

This case-control study was conducted in the Amol, which is in the north of Iran has the population of one million, from February 2011 to November 2011. The study is powered at 80% with a 2-sided 5% to achieve a statistically significance on a moderate standardized effect size of 0.4 (mean difference/ sd difference) between the Diabetics & controls with acute myocardial infarction on relevant blood samples. 1oo subjects per group were required randomly.

The case group had a history of at least five years of type 2 diabetes and consumption of oral hypoglycemic agents. The control group had no history of diabetes, with fasting blood sugar less than 126 mg/dl.

The study samples were hospitalized in cardiac care unit (CCU) of Imam Reza hospital and received routine treatments on AMI; 12^{cc} of patients' blood sample (2^{cc} of citrated blood and 10^{cc} for plasma separation) were prepared at 8 a.m. after 14 hours of fasting, and blood-containing tubes were immediately placed in the ice container and transported to the laboratory with 10 minutes time interval; the serum was then centrifuged to measure the CBC[1], triglycerides, cholesterol, LDL[2], HDL[3], ferritin, iron and TIBC[4].

All samples were tested in one laboratory by the same person using one kit and a specific system from the beginning to the end of the study. ELISA kits from Pishtaz Teb Zaman Manufacturing Research Company, the Biochemistry kits, and Darman Kav standard kits were applied for the measurement of ferritin and iron level, as well as TIBC measurement using the manual method respectively.

Patients with cardiomyopathy, hemolytic and megaloblastic anemia, alcoholism along with hepatic impairment, diagnosed hemochromatosis, Hodgkin's lymphoma, long and severe gastrointestinal bleeding, premenopausal women, bleeding disorders, and those in use of oral iron tablets during hospitalization were excluded from the study. [12]

Data were analyzed by descriptive statistics, chi-square, one-sample t-test, independent t-test, MANOVA, and logistic regression. In the crude odds ratio, all the independent variables were entered into the logistic regression (enter) model one by one, and in the adjusted odds ratio, all variables were simultaneously entered the backward stepwise regression model.

1- Complete blood count
2- Low density lipoprotein
[3] - High density lipoprotein
[4] - Total iron-binding capacity

A one-way between-groups multivariate analysis of variance was performed to investigate sex differences in diabetes type 2. Three dependent variables were used: ferritin, iron, and LDL. The independent variables were sex and diabetes. Preliminary assumption testing was conducted to check for normality, linearity, univariate and multivariate outliers, homogeneity of variance-covariance matrices, and multicollinearity, with no serious violations noted. P<0.05 was considered as a statistically significant level.

For ethical considerations, subjects were given the written informed consent, and no specific therapeutic or diagnostic procedure or additional cost was imposed to them; all the information obtained was kept confidentially and was not provided to any factual or legal reference. The study was approved by the ethics committee of Babol University of Medical Science.

Results

Regarding the results of the present study, 51.5% (N= 103) of patients were male. The mean age of male and female participants was 62.34 (SD=10.93) and 59.17 (SD=11.45) years respectively (p=0.04). The mean age of the study samples was 60.28 (SD=11.21) years in the case and 61.34 (SD=11.36) years in the control group respectively (p= 0.5). The case group consisted of 41 male and 59 female and the control group, 58 male and 42 female. The mean iron and ferritin level was respectively 79.78 (SD=32.24) mg/dl and 167.11 (SD=114.16) ng/dl in patients with a history of smoking, and 72.45 (SD=31.91) mg/dl and 171.74 (SD=114.16) ng/dl in those with no history of smoking (p=NS).

As shown in Table 1, there was a significant difference between gender (p=0.002), history of smoking (p <0.001), LDL (p=0.004), triglycerides (p=0.04), HCT[5] (p=0.05), MCH[6] (p=0.02), MCHC[7] (p=0.01) and type 2 diabetes.

Although ferritin level was 171.20 (SD=114.35) ng/dl in the case and 168.80 (SD=124.46) ng/dl in the control group, the difference was not statistically significant (p=0.8).

According to Table 1, the mean serum iron level was 25 mg/dl higher in the case than the control group (p <0.001).

As presented in Table 2, iron and ferritin levels of patients with MI were lower than the standard level in both genders (p<0.001).

Table 3 exhibits the correlation between independent variables and development of diabetes in logistic regression model. As the multivariate-adjusted model shows, the risk of diabetes development is 2.80 times higher in women than men and 4.03 times more in

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[5]- Haematocrit
[6] - Mean corpuscular haemoglobin
[7]- Mean corpuscular haemoglobin concentration

smokers than non-smokers. Based on percentile, the population was divided into four quarters, and regarding the results of adjusted logistic regression model, the risk of developing type 2 diabetes would be approximately 1.86 times higher per each quarter increase in the iron level (p<0.001).

According to MANOVA test results, There was a statistically significant difference between interaction effects of gender and diabetes on the combined dependent variables: F (3, 194) =3.18, p=.04; Wilks'Lambda=.95; partial eta squared=.04. When the results for the dependent variables were considered separately, the only difference to reach statistical significance using a Bonferroni adjusted alpha level of .037, was iron: F(1, 6027)=7.16, p=.008, partial eta squared=.03.

An inspection of the mean scores indicated that females with diabetes type 2 reported higher levels of iron (M=95.76, SD=39.34) than males (M=76.12, SD=25.61).

Discussion

Although the level of ferritin was not statistically different between the two groups, the serum iron level was higher in the case than the control, and the difference did not change even after exerting the effect of other variables.

Intracellular ferritin is made by smooth endoplasmic reticulum, while the rough endoplasmic reticulum plays the role in the production and secretion of plasma ferritin. Seventy percent of plasma ferritin produced binds to glycogen prior to secretion. Albeit the plasma ferritin has smaller amount of iron compared to intracellular ferritin, there is a correlation between intracellular iron storage and iron secretion.[21]

According to the present research, in spite of the fact that diabetic patients develop myocardial infarction at an earlier age, the difference was not statistically established, which is in accordance with Funk[22] and Devon studies.[23]

Regarding the results of multivariate logistic regression, female gender increases the risk of developing type 2 diabetes to approximately 5.5 times. Other risk factors except diabetes lead to remarkable increment in MI development in males. However, women are less sensitive to the mentioned risk factors, and only the diabetes makes them severely susceptible to myocardial infarction; as a result, the odds ratio has been considerably increased among this group.

Although the actual mechanism of the effect of female estrogen hormone on diabetes is not well known according to Zhang et al. (2002), the biological mechanisms of exogenous estrogen application have been discussed on the process of developing diabetes as antagonistic role of estrogen on insulin, elevated level of growth hormone and glucocorticoids, and changes in glucose absorption from the intestine.[24] Scholl (2005) indicates that high ferritin levels (170ng/ml) in women augment the risk of developing type 2diabetes to three

times within 10 years, without any relation to other risk factors such as BMI, age, and race.[25]

Despite higher ferritin levels in the case than the control group, the difference was not statistically significant, which is consistent with some investigations[6, 18] and inconsistent with others[12, 24]. In a study by School et al. on 1023 pregnant woman, it was found that the chance of developing gestational diabetes is two-fold higher in those with highest percentile of serum ferritin in the first trimester of pregnancy, and the rate will be almost three-fold in the third trimester.[25] Our research contradictory result may be due to ethnic differences, accuracy of ferritin measurement, and/or gender effect.

Findings of the present study suggest that per each quarter increase in serum iron, the risk of developing diabetes increases to more than 90%, which is in agreement with some researches.[12, 26] Diabetes along with high iron level might have made the person susceptible to myocardial infarction.

In a study by Mert et al. (2005) for the comparison of 130 non-diabetic and type 2 diabetic patients with acute myocardial infarction, it has been concluded that although the mean iron level was higher in non-diabetic than diabetic patients, the difference was not statistically significant.[26] Such a discrepancy seems to be owing to allocation of more than 40% of postmenopausal women to type 2 diabetic group, while, men comprised 70% of diabetic samples in Mert study. The mean difference of patients' blood iron levels in one hand, and the same mean age of males and females participated in the present study and Mert investigation on the other can be indicative for the involvement of other factors such as differences in the extent of necrosis in myocardial muscle.

The exact mechanism of the effect of increased blood iron level on diabetes development is still unknown; nonetheless, three key mechanisms are believed to be involved: 1. Insulin deficiency, 2. Insulin resistance, and 3. Hepatic dysfunction.[15]

Through formation of oxygen free radicals and lipid peroxidation, iron catalyzes the cellular reactions leading to coronary artery stenosis and myocardial susceptibility to ischemia and eventually AMI.[26]

The results of this research revealed enhanced possibility of diabetes development with increasing LDL level. Khososi et al. (2005) have regarded plasma oxidized LDL as one of the deleterious vascular complications which takes place even in the presence of small amounts of iron.[27]

Cross-sectional design was one of the main limitations of our research project which did not allow researchers to investigate type 2 diabetes-associated causes and risk factors among the study participants. Small sample size can be regarded as the other constraints of the study. Therefore, it is recommended that subsequent studies be conducted with larger sample size and evaluation of more biomarkers.

Conclusion

Although some studies have put forward the effect of high ferritin levels on the pathogenesis of type 2 diabetes and AMI, our findings have not supported the hypothesis of ferritin impact on type 2 diabetes development in patients with AMI, and only the role of iron, as a powerful antioxidant, underlies development of type 2 diabetes, on which further comprehensive studies seem to be needed.

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Table1. Comparison of the mean, standard deviation and proportion of demographic variables between participants with and without type 2 diabetes

Study variables		Case	Control	P value
Age		60.28 (SD=11.21)	61.34 (SD=11.36)	0.5
Gender	Male	41	58	
	Female	59	42	0.002
Smoking	Yes	51	24	
	No	49	76	<0.001
Cholesterol (mg/dl)		188.65 (SD=36.78)	196.87 (SD=49.25)	0.1
Triglyceride (mg/dl)		119.22 (SD=38.40)	163.64 (SD=38.40)	0.04
LDL (ng/dl)		133.44 (SD=36.58)	118.91(SD=33.84)	0.004
HDL (ng/dl)		43.44 (SD=7.83)	43.03 (SD=10.50)	0.7
Hb		13.76 (SD=1.78)	13.56 (SD=2.72)	0.5
HCT		41.70 (SD=4.67)	39.85 (SD=4.56)	0.005
MCV		89.26 (SD=6.37)	88.43 (SD=7.19)	0.3
MCH		29.90 (SD=2.82)	28.91 (SD=3.19)	0.02
MCHC		33.05 (SD=1.31)	32.37 (SD=2.32)	0.001
Ferritin (ng/dl)		171.20 (SD=114.35)	168.80 (SD=124.46)	0.8
Iron (mg/dl)		87.71 (SD=35.58)	62.70 (SD=22.27)	<0.001
TIBC		34.99 (SD=42.25)	352.38 (SD=56.57)	0.3

Table 2: Comparison of the mean and standard deviation of serum ferritin and iron levels with maximum standard level in 200 patients with AMI

	Results			
Variable		Mean (SD)	95% CI	P
Ferritin	Female [1]	169.33 (108.70)	71-115	<0.001
	Male [2]	170.63 (128.88)	127-177	<0.001
Iron	Female [3]	84.35 (36.09)	58-72	<0.001
	Male [4]	66.59 (25.23)	88-98	<0.001

Abnormal: 1. (>263ng/dl), 2. (>323ng/dl), 3. (>263ng/dl), 3. (>150mg/dl), 4. (>160mg/dl)

Table 3: Predictors diabetes in patients with AMI

Logistic Regression	unadjusted			adjusted		
Variable	OR	95%CI	P	OR	95%CI	P
Gender Male/Female[£]	2.67	1.39 - 5.13	0.003	2.80	1.35-5.28	0.006
History of smoking Yes[£]/No	3.29	1.80-6.02	<0.001	4.03	1.94-8.36	<0.001
HCT	1.09	1.02-1.16	0.006			
Serum iron[¥]	2.07	1.56-2.75	<0.001	1.86	1.35-2.56	<0.001
LDL	1.01	1.005-1.03	0.005			
MCH	1.12	1.01-1.23	0.02			
MCHC	1.23	1.03-1.46	0.01			

[£] Reference
[¥] Blood iron level was divided into four quarters based on percentile and odd ratio was calculated. All variables with p<0.1 in crude analysis were entered in the adjusted model in step 1; then using backward stepwise method, only significant variables were selected in the final adjusted model.