Özgün Çalışma / Original Article

Ankara Eğt. Arş. Hast. Derg., 2024 ; 57(1) :20-24
DOI: 10.20492/aeahtd.1377001

Diyabetik Kadınlarda Serum Hem Oksijenaz-1 (Ho-1) Enziminin Glisemik Kontrol ile İlişkisi

Association of Heme Oxygenase-1 (Ho-1) Enzyme Levels with Glycemic Control in Diabetic Women

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ABSTRACT

AIM: The rapidly increasing prevalence of diabetes has made it a significant global health issue. While there are existing treatment options, there is a need for innovative approaches to treatment. The heme molecule broken down by heme oxygenase-1 produces CO, bilirubin, and Fe/ferritin. Animal experiments have shown that products resulting from heme oxygenase-1 induction attenuate inflammation, oxidative stress and apoptosis and reduce hyperglycemia. We aimed to investigate whether serum heme oxygenase-1 has an effect on glycemic status in women with diabetes.

MATERIAL AND METHOD: Sixty-three female patients (31 diabetic and 32 control) who applied to the outpatient clinic were included in the study. The study excluded patients with acute and chronic renal failure, patients with acute or chronic liver disease, patients with acute infection, patients with a body mass index (BMI) of ≤18 kg/m2 or ≥35 kg/m2, and patients younger than 18 years and older than 65 years.

RESULTS: Serum ferritin was significantly higher in the patient group compared to the control group (p = 0.028). Serum total bilirubin and serum heme oxygenase-1 were similar in the two groups (p = 0.260, p = 0.426, respectively).

CONCLUSION: In our study, serum ferritin were significantly higher in the diabetes group than controls, supporting a possible role for ferritin in diabetes pathogenesis. Nevertheless, no considerable differences were observed in serum total bilirubin and serum heme oxygenase-1 between the groups. This suggests that the relationship between serum ferritin and glycemic parameters may not be directly associated with the breakdown of heme molecules by heme oxygenase-1.

Keywords: heme oxygenase-1, diabetes mellitus, ferritin, total bilirubin

ÖZET

AMAÇ: Diyabetin hızla artan prevalansı, onu önemli bir küresel sağlık sorunu haline getirmiştir. Mevcut tedavi seçenekleri olsa da, tedavide yenilikçi yaklaşımlara ihtiyaç vardır. Hem molekülünün hem oksijenaz-1 ile parçalanması sonucu CO, bilirubin ve Fe/Ferritin ortaya çıkar. Yapılan hayvan deneyleri, hem oksijenaz-1 indüksiyonu sonucu ortaya çıkan ürünlerin inflamasyon, oksidatif stres ve apoptozu hafiflettiği ve hiperglisemiyi azalttığı gösterilmiştir. Bizde, diyabetli kadınlarda glisemik durum üzerine serum hem oksijenaz-1'in etkisinin olup olmadığını incelemeyi amaçladık.

GEREÇ VE YÖNTEM: Polikliniğe başvuran 63 kadın hasta (31'i diyabetik, 32'si kontrol) çalışmaya dahil edilmiştir. Çalışmaya akut ve kronik böbrek yetmezliği olan hastalar, akut veya kronik karaciğer hastalığı olan hastalar, akut enfeksiyonu olan hastalar, vücut kitle indeksi (VKİ) ≤18 kg/m2 veya ≥35 kg/m2 olan hastalar ve 18 yaşından küçük ve 65 yaşından büyük hastalar dahil edilmemiştir.
BULGULAR: Serum ferritin hasta grubunda kontrol grubuna kıyasla anlamlı olarak daha yüksekti (p = 0.028). Serum total bilirubin ve serum hem oksijenaz-1 iki grupta benzerdi (sırasıyla p = 0.260, p = 0.426).

SONUÇ: Çalışmamızda diyabet grubundaki serum ferritin düzeyinin kontrol grubuna göre anlamlı ölçüde daha yüksek olması ferritinin diyabet patogenezindeki olası rolünü desteklemektedir. Bununla birlikte, serum total bilirubin düzeyleri ve serum hem oksijenaz-1 düzeylerinde gruplar arasında önemli bir fark gözlenmemiştir. Bu durum, serum ferritin ile glisemik parametreler arasındaki ilişkinin doğrudan hem moleküllerinin hem oksijenaz-1 tarafından parçalanması ile ilişkili olmayabileceğini düşündürmektedir.

Anahtar Kelimeler: hem oksijenaz-1, diabetes mellitus, ferritin, total bilirubin

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Makale geliş tarihi / Submitted: Ekim 2023 / October 2023

Makale kabul tarihi / Accepted: Şubat 2024 / February 2024

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INTRODUCTION

Diabetes is characterized by elevated levels of glucose, which result in increased production of reactive oxygen species through a number of mechanisms. The resultant oxidative stress plays a significant part in the etiology of diabetes[1]. There are many studies on the place of antioxidants in the treatment of diabetes. Therefore, an approach has emerged that antioxidants can be part of the therapeutic process[2]. It has been shown that upregulation of the HO system in different diabetic models increases insulin secretion from pancreatic beta cells and reduces hyperglycemia[3].

In a prevalence study, it is estimated that 463 million people in 2019 had diabetes mellitus (DM) (global prevalence 9.3%). In 2030, this count is anticipated to rise to 578 million (global prevalence 10.2%)[4]. Despite advances in treatment, DM remains a global problem. Novel metabolic pathways need to be aimed to build a new pharmacological treatment to restore glucose homeostasis and reduce the metabolic sequelae[5]. Heme oxygenase-1 (HO-1) is activated when it forms a complex with heme. As a consequence of HO-1 activity, heme protein is broken down and bilirubin, carbon monoxide and ferrous iron are formed[6]. It has been demonstrated that the products released following the breakdown of the heme protein by the HO-1 enzyme have anti-inflammatory features[7]. Animal studies have found positive effects of HO-1 on wound healing and vascular complications in diabetic subjects[8].

Heme oxygenase has multiple forms. HO-1 is its inducible form[9]. Many proteins, for example myoglobin, cytochromes, and hemoglobins utilize heme as cofactors. HO-1 activity degrades heme into biliverdin, carbon monoxide (CO), and iron. The biliverdin reductase system rapidly converts biliverdin to bilirubin[10]. Iron released from the HO-1 activity is captured through ferritin. In this way, the toxicity of iron to the tissues is prevented[11]. Ferritin is a cytoprotective molecule. Increased ferritin synthesis due to HO-1 activity potentiates the HO-1-mediated cytoprotection and makes cells more resistant[12]. In addition, bilirubin is an endogenous substance in the serum that functions as an antioxidant and anti-inflammatory agent[13]. Furthermore, the HO system and associated products (ferritin, bilirubin and carbon monoxide) have been demonstrated to alleviate inflammation, oxidative stress and apoptosis as well as to reduce hyperglycemia[14].

It has been claimed that the SM genotype of the HO-1 gene in humans may be related with type 2 DM and chronic inflammatory diseases[15]. A meta-analysis suggests that individuals with longer (GT) (n) repeats within the HO-1 gene promoter may be at a greater risk of developing type 2 diabetes mellitus[16]. Chen et al. found that HO-1 gene polymorphism (longer (GT)(n) repeats) may predispose diabetic patients to coronary artery disease[17]. Furthermore, research indicates a correlation between the development of albuminuria in patients with type 2 DM and polymorphism of the HO-1 gene, specifically the T(-413)A single nucleotide polymorphism[18]. Another finding in the literature was that HO-1 concentrations were lower in patients with proliferative diabetic retinopathy than in those without[19].

There are animal and cell culture experiments showing that induction of HO-1 activity may be beneficial in glycemic control in diabetes[20]. There are also studies showing that HO-1 induction has favorable effects on diabetic complications for instance retinopathy and neuropathy[21,22]. As a result, we aimed to investigate the correlation between hem oxygenase-1 and glycemic state in female individuals with diabetes.

MATERIAL AND METHOD

Thirty-one DM patients and thirty-two healthy individuals with similar characteristics were included in the study from those who visited the outpatient clinic. The weight of the patients was measured with a digital weighing device and their height was measured with a standing height meter. The blood test results of the participants at the time of application were evaluated. The study excluded patients with acute and chronic renal failure, patients with acute or chronic liver disease, patients with acute infection, patients with a body mass index (BMI) of ≤18 kg/m2 or ≥35 kg/m2, and patients younger than 18 years and older than 65 years. In addition, patients with microvascular and macrovascular diabetic complications were excluded. Serum ferritin and total bilirubin were analyzed to evaluate the effects of serum HO-1 directly or through its degradation products. In addition, serum high sensitive-CRP (hsCRP) were analyzed to evaluate whether serum HO-1 has an effect on glycemic control through anti-inflammatory effect. Laboratory analyses were performed on venous blood samples obtained after 12 hours of fasting. The serum HO-1 was measured using the "Biotek Instruments Inc. ELX50 and Biotek Instruments Inc. ELX800" devices.

Statistical analysis was performed using the SPSS 16.0 for Windows (SPSS, Inc.; Chicago, USA) package program. For statistical analysis, in the descriptive findings section, categorical variables were evaluated with numbers and percentages, while continuous variables were presented with mean ± standard deviation and median (minimum and maximum value). Continuous variables were evaluated according to a normality assessment with Kolmogorov-Smirnov and Shapiro-Wilk tests. If continuous variables were not normally distributed, they were compared with the nonparametric Mann-Whitney U, and if they were normally distributed, they were compared with the T Test in parametric independent groups. The pearson Correlation analysis test was used in the evaluation of the relationships between blood values, according to their conformity to the normal distribution. Statistical tests with a p value <0.05 were considered significant. Ethics committee approval, dated 24.08.17 and numbered 5492, was obtained from the Turkish Ministry of Health Ankara Training and Research Hospital Ethics Committee for the study.

RESULTS

There was no significant difference between the groups in terms of mean age and BMI. (respectively p = 0.06, p= 0.15). Serum HO-1 and serum total bilirubin concentrations were not different between groups (respectively p=0.40, p=0.26) Serum ferritin were significantly elevated in diabetic patients compared to controls (p = 0.02). The blood test results obtained from the patient and control groups are shown in Table 1.

Table 1: Demographic and biochemical characteristics of both groups.

	Patients (n=31)	Controls (n=32)	p value
Age	53,16 ± 6,13 yıl	49,22 ± 10,00 yıl	0,064
BMI	28,76 ± 3,12 kg/m2	27,52 ± 3,75 kg/m2	0,159
HO-1	2,99 ± 1,99 ng/ml	3,39 ± 1,94 ng/ml	0,426
Total bilirübin	0,54 ± 0,22 mg/dl	0,79 ± 1,21 mg/d	0,260
Ferritin	39,46 ± 37,80 ng/ml	22,51 ± 17,31 ng/ml	0,028
FPG	163,00 ± 68,54 mg/dl	90,81 ± 6,29 mg/dl	<0,001
PPG	235,33 ± 90,03 mg/dl	110,83 ± 22,05 mg/dl	<0,001
HbA1c	%7,96 ± 2,01	%5,46 ± 0,38	<0,001
Sedimentation	20,97 ± 12,80 mm/sa	11,94 ± 9,32 mm/sa	0,002
hsCRP	4,24 ± 2,86 mg/L	2,43 ± 2,83 mg/L	0,014
Hemoglobin	13,18 ± 1,46 g/dl	13,11 ± 1,19 g/dl	0,840
WBC	7,70 ± 1,48 *10^3/mm^3	7,11 ± 1,95 *10^3/mm^3	0,189
Platelets	292,23 ± 52,08 *10^3/mm^3	267,50 ± 57,60 *10^3/mm^3	0,079
Neutrophil	1,91 ± 0,70 *10^3/mm^3	2,22 ± 1,00 *10^3/mm^3	0,164
Lymphocytes	3,21 ± 0,68 *10^3/mm^3	2,95 ± 0,69 *10^3/mm^3	0,140
Creatinine	0,77 ± 0,10 mg/dl	0,80 ± 0,10 mg/dl	0,289
Urea	1,91 ± 1,82 mg/dl	2,38 ± 1,68 mg/dl	0,298
Albumin	4,45 ± 0,42 mg/dl	4,34 ± 0,33 mg/dl	0,247

BMI: Body mass index, HO-1: Heme oxygenase-1 FPG: fasting plasma glucose; PPG: postprandial glucose; HbA1c: glycated haemoglobin hsCRP: high-sensitivity C-reactive protein WBC: white blood cells; LDL: low density lipoprotein; HDL: high density lipoprotein

In the study population, no correlation was found between serum HO-1 and glycemic parameters. Ferritin was positively correlated with hemoglobin A1C (HbA1c) and fasting and postprandial glucose. Moreover, high-sensitivity C-reactive protein (hsCRP) was positively correlated with BMI, fasting and postprandial glucose (Table 2).

Table 2: Correlation Analysis Results

		HO-1	Ferritin	Total bilirubin	HbA1c	hsCRP	FPG	PPG	BMI
HO-1									
Ferritin	r	-0.02							
	p	0.84							
Total bilirubin	r	0.21	0.08						
	p	0.09	0.49						
HbA1c	r	0.08	**0.48****	-0.06					
	p	0.49	**<0.001**	0.61					
hsCRP	r	-0.09	0.22	0.01	**0.48****				
	p	0.48	0.07	0.92	**<0.001**				
FPG	r	-0.01	**0.47****	-0.05	**0.83****	**0.43****			
	p	0.90	**<0.001**	0.65	**<0.001**	**<0.001**			
PPG	r	-0.03	**0.48****	-0.05	**0.74****	**0.46****	**0.84****		
	p	0.77	**<0.001**	0.68	**<0.001**	**<0.001**	**<0.001**		
BMI	r	-0.06	-0.16	0.09	0.10	**0.29***	0.02	0.09	
	p	0.62	0.18	0.47	0.39	**0.02**	0.87	0.47	

** The correlation is significant at the 0.01 level (2-tailed). * The correlation is significant at the 0.05 level (2-tailed). HO-1: Heme oxygenase-1 FPG: fasting plasma glucose; PPG: postprandial glucose; HbA1c: glycated haemoglobin hsCRP: high-sensitivity C-reactive protein

DISCUSSION

Serum ferritin was significantly higher in the patient group compared to the control group. Serum total bilirubin and serum heme oxygenase-1 were similar in both groups. Serum ferritin correlated with glycemic parameters, whereas serum total bilirubin and HO-1 did not. In addition, there was no difference in hsCRP levels between the groups.

There are studies in the literature that detect a genetic polymorphism-based relationship between HO-1 and DM[15,16]. In this study, serum HO-1 was used instead of genetic polymorphism and it did not differ between groups. The relationship between HO-1 and DM may be due to differences in genetic rather than serum level. This dissimilarity may be the reason why there was no significance for HO-1 s in our study. There are animal and cell culture experiments showing that activation of HO-1 may be useful in glycemic control in DM[20,23]. In our study, no correlation was found between indicators of poor glycemic control (FPG, PPG, and HbA1c) and serum HO-1 (Table 2). In our study, unlike the studies mentioned above, serum HO-1 in humans were examined instead of animal models or cell culture. In addition, the results of HO-1 induction were examined in the above-mentioned studies. All of these factors can be responsible for the diversity between our study and the aforementioned studies. A polymorphism of the enzyme HO-1 was found to play a part in the occurrence of albuminuria in patients with DM in the study by Lee et al[18]. Castillo et al. found that HO-1 was protective against retinopathy due to hyperglycemia and oxidative stress[24].

It has been suggested that elevated serum hsCRP may be an indicator of the low-grade inflammation that underlies the development of type 2 DM[25]. Furthermore, several researchers have suggested that elevated concentrations of hsCRP are associated with diabetic complications in diabetic patients[26–28]. In our study, hsCRP and erythrocyte sedimentation rate, markers of inflammation, was significantly increased in the DM group compared to the control group, similar to the literature. However, as there was no significant difference between the groups, this result could not be related to HO-1. Moreover, it has been suggested that HO-1 may be beneficial on neuron apoptosis and neuron damage responsible for diabetic neuropathic pain[22]. These findings support that HO-1 can also be linked with DM complications. The lack of classification according to complications in our patient group may have had an impact on the results.

It has been suggested that serum ferritin have an effect on carbohydrate and lipid metabolism and cause insulin resistance[29]. In addition, it has been determined that hyperferritinemia, which develops without iron load, is associated with insulin resistance[30]. Moreover, the association between serum ferritin and diabetes has been described previously[31]. Consistent with the literature, a positive correlation was found between ferritin and glycemic dysregulation (FBG, PPG, HbA1c) in our study (Table 2). Furthermore, seum ferritin of the DM group was significantly higher than that of the control group. Miguel et al. claimed that the relationship between ferritin elevation and type 2 DM may be due to HO-1 activity[32]. In another study, it was determined that the polymorphism of the HO-1 gene may be related with type 2 DM both with and without ferritin elevation[15]. The association between ferritin and diabetes detected in our study could not be associated with the serum HO-1. The low number of patients and the inclusion of only female patients in the study may have contributed to this result.

A cross-sectional study involving 38,641 adults found that low serum total bilirubin were associated with a risk of developing DM[33]. However, there are also studies suggest that serum total bilirubin do not correlate with the development of DM[34,35]. In addition, some studies have claimed that there is a reverse association between total bilirubin concentration and HbA1c[36,37]. Another point to consider is that there have been inconsistent results between studies that have examined the relationship between total bilirubin and FBG [38–41]. In our study, no correlation was found between total bilirubin and glycemic parameters (FBG, PPG, HbA1c) (Table 2). Furthermore serum total bilirubin were not statistically different between groups. Findings on this subject are controversial. Differences in study design and racial differences may have contributed to these results.

CONCLUSION

In this study, although there was a significant difference in serum ferritin between the groups, there were no statistically significant differences between the groups in serum total bilirubin and serum HO-1. The relationship between serum ferritin and glycemic parameters is consistent with the studies in the literature. The role of ferritin in the pathogenesis of diabetes seems to be a topic worthy of further investigation. However, the fact that no relationship was found between serum HO-1 and DM is a different result from the studies in the literature. This makes it difficult to establish a relationship between serum HO-1 and serum ferritin. Further research is needed to define the association between ferritin, total bilirubin, HO-1 and diabetes.

ACKNOWLEDGEMENTS

The authors have no conflicts of interest to declare. The authors declared that this study has received no financial support.

AUTHOR CONTRIBUTIONS: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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