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Studying the Role of Heme Oxygenase-1 in Obese Patients

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Abstract

Heme oxygenase-1(HO-1) is an enzyme that catalyzes and breaks down heme molecules into biliverdin, free iron, bilirubin, and carbon monoxide. This study aims to investigate the levels and role of (HO-1) in obese patients against oxidative stress and its relationship to obesity. The study included 139 samples: (84 obese and 55 non-obese persons). Both study groups were divided into four groups based on their Body Mass Index (BMI). Blood sample was collected from obese persons and control groups (men and women) at AL-Yarmouk Hospital and the National Diabetes Research Center at the period between December-2022 until June-2023. Some biochemical parameters were measured for all studied groups, which include: Determining of HO-1 levels in serum by using the ELISA-technique, lipid profile and fasting serum glucose (FSG) assessed enzymatic. BMI levels were found to be increased significantly in obesity class II group, obesity-class I group, and overweight group compared with normal weight groups. Also, the results showed that waist hip ratio (WHR) was significantly increased ($p \leq 0.05$) in groups obesity class II, obesity class I, and overweight group compared with normal weight group. Also, the results showed that the HO-1 levels were higher in obese-class I patients and obese-class II group compared with normal-weight and overweight groups. The statistical analysis displayed that, the level of HO-1 is associated negatively with BMI in normal weight group (G1), while positively with the obesity-class II, obesity-class I, and overweight groups. We conclude from this study that the body's first line of defense against oxidative stress attack is HO-1. This cell-protective enzyme reduces oxidative stress and is vital for controlling lipogenesis, which is crucial for the development of metabolic diseases and its complications.

Keywords: Body mass index, FSG, Heme oxygenase -1, Obesity, Waist hip ratio.

Introduction

One of the major risks to population health is obesity. The World Health Organization (WHO) states that between 1975 and 2016, the prevalence of obesity approximately quadrupled globally. In 2016, 13% of adults worldwide had obesity and 39% of people were overweight[1].The (BMI) is a method of evaluating an adult's weight status based on the weight in kilograms (kg) divided by the square of height in meters (m^2), is the most frequently used indicator in social science research to assess obesity[2-3]. Further assessment of the distribution of body fat can be done by evaluating the waist circumference (WC) and the (WHR) which represent what is known as central or abdominal obesity, both of which are

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significant predictors in young and middle-aged adults as compared to older people and those with low BMI[4]. The worldwide obesity epidemic has contributed to cardiovascular issues such as hypertension, dyslipidemia, and vascular dysfunction. Many of the vascular issues related to obesity are brought on by adipocyte dysfunction and subsequent endothelial degradation[5]. The adipose tissue (AT) is the most efficient site for storing extra fat calories. In humans, adipose tissue, a substantial and active endocrine organ that is involved in energy storage, accounts for between 20 to 25% of total body mass [6]. The only cells in the body that are designed to securely store significant amounts of fat are the fat cells of adipose tissue. Adipose tissues (mainly white adipose tissue) are distributed in the subcutaneous fat (located under the skin) and the visceral fat (located intra-abdominally, adjacent to internal organs)[7]. When excessive amounts of nutrients are consumed, the fat tends to build up in the visceral and subcutaneous depots, enlarging these depots through adipocyte cell hypertrophy and hyperplasia, and making them unhealthy [8]. HO-1 promotes browning of white adipose tissues (WAT) and prevents the development of inflammatory, big WAT HO-1 improves mitochondrial quality control and reduces myocyte death in the myocardium[9]. One enzyme that has proved crucial in managing metabolic diseases is heme oxygenase (HO). The isozymes HO-1 (inducible form) and HO-2 are present in humans (constitutive form). In terms of their processes, cofactor and substrate needs, and susceptibility to activation or inhibition by artificial metalloporphyrin, in which the iron atom at the center of heme has been substituted by other elements including tin, zinc, cobalt, and chromium, isozymes are comparable .By generating equimolar amounts of carbon monoxide (CO), iron, and biliverdin, the pro-oxidant HO which is also a strong inducer of HO-1contributes to the breakdown of heme .Biliverdin reductase subsequently transforms the biliverdin into bilirubin. Superoxide dismutase and catalase are two examples of antioxidant enzymes that are improved when HO-1 is activated[10].The secretion of large amounts of HO-1increases the resistance of cell injury by heme. Numerous mechanisms, including a decrease in the concentrations of cellular heme-dependent proteins that raise oxidative stress and a rise in the production of the antioxidant bilirubin, have been implicated in the well-documented protective role of HO-1 against the development of diabetes and metabolic disease. In addition to the role of heme iron in increasing, the accumulation of fats generated in adipose / visceral tissues[11]. This study aims to investigate the levels and role of (HO-1) in obese patients against oxidative stress and its relationship to obesity.

Materials and Methods

Patients and Control

This study was conducted at the College of Science for Women, University of Baghdad. The samples were collected from AL- Yarmouk Teaching Hospital and the National Center for Diabetes Research , 139 samples were collected , then they were divided according to the BMI according to the National Institute of Health (NIH)and the World Health Organization (WHO),into two groups, the first group includes healthy people (55) and includes 32 persons with normal weight (G1) and 23 persons with overweight (G2), and the second group includes 84 obese patients , and include 43 obese class I patients (G3) and 41 obesity class II patients (G4)]. The BMI was calculated using the following equation: Weight in kg/m^2 [12]. 7 ml of the venous blood was collected for each participant of men and women through a 10 ml syringe, the blood was then put into a gel tube, and then the tube was left for separation, then centrifuged at 3000 rpm for ten mints to obtain serum. The blood serum was used for the check of blood glucose levels and lipid profile that measured manually by humane Germane kit. The residual serum was frozen at -20°C for assessment of Heme oxygenase -1(HO-1) by ELISA (My BioSource, USA).

Statistical Analysis

The Statistical Packages for Social Sciences (SPSS Inc., Chicago U, S, A) version 26 was used to analyze the data. The data was presented as (mean ± SE). ANOVA test for difference between three independent variables, Tukey-test, ROC curve and correlation coefficient (r) between parameters.

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Estimation by analyzing for linear regression was employed in the statistical test. The statistical significance was determined by the probability value, which was acknowledged as significant at $p \leq 0.05$ and non-significant at $p > 0.05$.

Inclusion Criteria

Subjects aged 35 to 65 years' old and free of disease.

Obese male and female.

Exclusion Criteria

Patients with any metabolic disease or diabetes mellitus or chronic diseases were excluded from this study.

Results and Discussion

The age for all studied groups in the current study was matched. The body mass index (BMI) and WHR exhibited a significant difference ($p \leq 0.05$) between the studied groups, as shown in the (mean ± SE) of BMI in Table1.

Table 1. Comparison of biochemical parameters between studied groups.

Groups Parameters	Normal weight Group (G1) No. (32)	Overweight Group (G2) No. (23)	Obesity class I Group(G3) No. (43)	Obesity class II Group(G4) No. (41)	P-value
Age (year)	46.68 ± 1.58 [a] (46.5)	48.13± 2.13 [a] (46)	46.65 ± 1.32 [a] (45)	46.66± 1.30 [a] (46)	0.915
BMI (kg/m^2)	23.10 ± 0.28 [c] (23.4)	26.40 ± 0.22 [d] (26)	32.03 ± 0.26 [a] (31.9)	38.83± 0.44 [b] (38)	0.0001**
Waist/Hip ratio	0.88 ± 0.01[a] (0.89)	0.93 ± 0.01 [ab] (0.94)	0.92 ± 0.02[ab] (0.90)	1.01 ± 0.03 [b] (0.96)	0.008**

The data were shown as Mean± SE (Median)

a, b, c, d are latter's refer to significant or non-significant between groups, in which the group have the same later are non-significant ,while the groups have different later are significant.

Also, lipid profile had significant value with obese patients in LDL-C and non-significant values with other parameters as shown in Table 2, overall obese participants had averages of LDL higher than those of non-obese participants. the mean values ± SE were [(83.94 ± 8.95[ab]) (112.93 ± 10.12 [b]) (99.74 ± 6.63 [ab]) (80.42 ± 7.47 [a]) respectively and $p \leq 0.05$.

Table 2. Comparison between patients and Control groups in lipid profile and Atherogenic

Groups Parameters	Normal weight Group (G1) No. (32)	Overweight Group (G2) No. (23)	Obesity class I Group(G3) No. (43)	Obesity class II Group(G4) No. (41)	P-value
FSG (mg/dL)	99.11 ± 2.69 [a] (97.85)	98.45 ± 3.04 [a] (96)	98.29 ± 3.13 [a] (94)	94.36 ± 2.85 [a] (90.5)	0.644
TC (mg/dL)	160.2 ± 10.34 [a] (153)	186.62 ± 10.5 [a] (178.7)	178.84 ± 41.10 [a] (184)	160.35 ± 7.04 [a] (162)	0.073
TG (mg/dL)	174.61 ± 16.89 [a] (156)	151.90 ± 15.15 [a] (156)	173.98 ± 16.22 [a] (153)	170.01± 14.98 [a] (151)	0.811
HDL-C(mg/dL)	42.70 ± 1.47[a] (43.8)	43.30 ± 1.82 [a] (43.1)	44.30 ± 1.184 [a] (44.5)	45.92 ± 1.72 [a] (45.6)	0.449
LDL-C (mg/dL)	80.42 ± 7.47 a (75.4)	112.93 ± 10.12 [b] (110.7)	99.74 ± 6.63[ab] (103)	83.94 ± 8.95[ab] (89.4)	0.033*
VLDL-C (mg/dL)	34.0± 2.99 a (30.2)	30.38 ± 3.03 [a] (31.2)	34.79 ± 3.24 [a] (30.6)	34.92 ± 3.37[a] (31.2)	0.811

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Atherogenic index	0.55 ± 0.05[a] (0.56)	0.50 ± 0.04[a] (0.53)	0.51± 0.04 [a] (0.56)	0.50± 0.04[a] (0.55)	0.889

Data were presented as Mean ± SE (Median)

a, b, c, d are latter refer to significant or non-significant between groups, in which the group have the same later are non-significant, while the groups have different later are significant.

The mean ± SE values of HO-1 (ng/mL) for all the studied groups in the current study were recorded in Table 3. The table shows a significant difference between G1, G2, G3, and G4 groups with p ≤0.05 difference in HO-1 levels between studied groups. The HO-1levels were very high in the obesity class I (G3) group compared with the G1, G2, and G4 groups, The Tukey-test between groups G2 and G3 showed p=0.05 while between G1 and G4 groups (p>0.05).

Table 3. Comparison between patients and Control groups in Hem oxygenase.

Groups Parameters	Normal weight Group (G1) No. (32)	Overweight Group (G2) No. (23)	Obesity class I Group(G3) No. (43)	Obesity class II Group(G4) No. (41)	P-value
Heme oxygenase(ng/mL)	1.88 ± 0.04[ab] (1.94)	1.75 ± 0.05 [a] (1.77)	2.06 ± 0.09 [b] (1.99)	1.95 ± 0.04 [ab] (1.89)	0.044*

The data were shown as Mean ± SE (Median)

a, b, c ,d are letters refer to significant or non-significant between groups, in which the group have the same later are non-significant ,while the groups have different later are significant .

Table 4, shows correlation of different parameters levels with HO-1 in obese patients and control groups. The results showed that there was strong negative correlation between HO-1 with BMI, in normal weight group G1, while positive significantly correlation with G2, G3, and G4. Also, significantly positive with LDL in G3 and G4, also significantly positive with TG in G3 using Pearson correlation at p≤0.05.

Table 4. Correlation coefficient between difference parameters with HO-1

		HO (ng/ml)			
		Normal weight Group (G1) No. (32)	**Overweight Group (G2) No. (23)**	**Obesity class I Group (G3) No. (43)**	**Obesity class II Group (G4) No. (41)**
Age (years)	R	-0.28	-0.173	-0.203	0.262
	P	0.06	0.276	0.264	0.226
BMI (kg/m2)	R	-0.317*	0.66**	0.425*	0.613**
	P	0.038	0.0001	0.015	0.001
Waist/Hip ratio	R	-0.027	0.145	-0.047	0.151
	P	0.862	0.364	0.794	0.491
FBG (mg/dL)	R	-0.037	0.037	0.183	0.318
	P	0.812	0.813	0.314	0.138
TG(mg/dL)	R	-0.146	0.112	0.401*	0.284
	P	0.349	0.485	0.022	0.188
TC(mg/dL)	R	0.045	0.002	0.118	-0.145
	P	0.774	0.989	0.517	0.507
HDL-C(mg/dL)	R	-0.029	0.079	0.085	-0.419*
	P	0.851	0.620	0.642	0.046
LDL-C(mg/dL)	R	-0.154	0.086	0.386*	0.415*
	P	0.321	0.590	0.028	0.048

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VLDL-C(mg/dL)	R	0.045	0.002	0.118	-0.145
	P	0.774	0.989	0.517	0.507
Atherogenic index	R	0.101	0.047	0.030	-0.055
	P	0.519	0.767	0.866	0.800

*Correlation is significant at the 0.05 level.
**Correlation is significant at the 0.01 level.

The value of area under the ROC curve for HO-1 in obese person groups G3, G4 was 0.611. Also, the cut off value for HO-1 was >80.36. The higher sensitivity and specificity were estimated for OH-1 (91.83 %, 26.7 %) respectively in obese patients, Fig. 1.

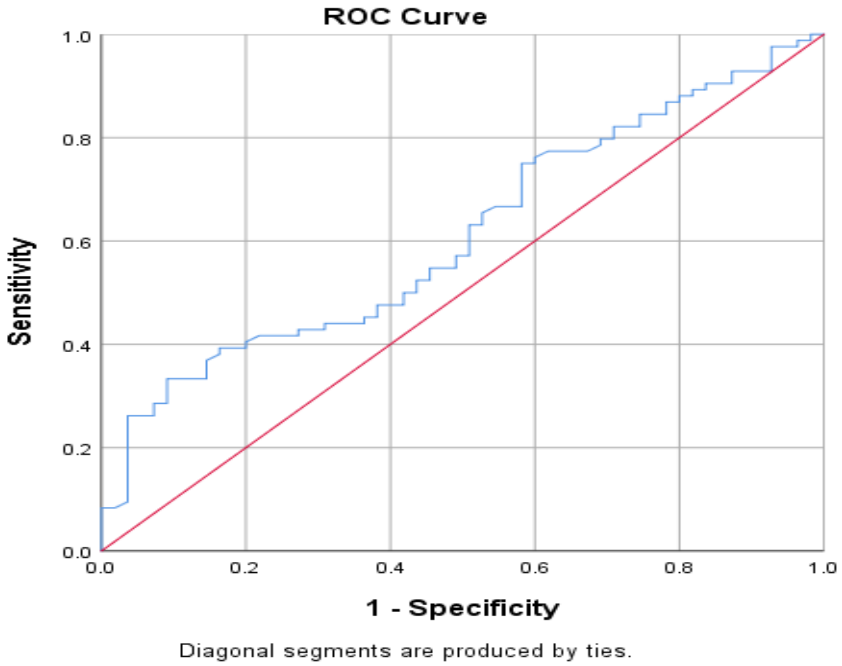

Figure 1. ROC curve analysis of HO-1 for patients and control groups

Discussion

The present study showed high significance in BMI and WHR in the study groups (P≤0.05), the BMI refers to weight gain can be caused by increasing muscle mass, bone density, or fat mass and WHR refers to the more belly fat there is, the more likely it is that you may acquire diseases like high cholesterol, diabetes, high blood pressure, or atherosclerosis. This agrees with Nadeem et al., who showed that BMI and WHR can be used to identify metabolic disorders in clinical and epidemiological investigations since they are straightforward and non-invasive, [13]. Other studies on German adult men and women, as well as Australian adults aged 20 to 69, found a high association between WC and BMI and a poor association between WHR and BMI[14].Also, this study agrees with Tutunchi et al.,[15]. as it also accords with the previous studies and our findings. These studies support our findings that WHR exhibited significant differences between subgroups but WHR revealed no significant difference between obese class I and obese class II, as seen in Table 1. The results also agree with Janjić, J.[16]. Due concerning variations in body composition and the fact that obesity significantly affects body mass index, various body fat percentage (BF%) metrics produce more accurate result. The result showed significance values with LDL- C, and non-significance values with other parameters, during times of high oxidative stress, a higher rate of reactive oxygen species (ROS) production in the mitochondria can lead to the formation of oxidized LDL. This result agrees with a

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study by Hazart J *et al*.,[17], who showed that there is non-significance with (HDL-c, TG, TC, VLDL. This also agrees with other study by Malaguarnera L, *et al*., [18], who found that there is a significant relationship between obesity and high bad fats (LDL), which causes stimulation of large amounts of enzyme OH-1 secretion in endothelial cells, smooth muscles, and connective tissue. The current study showed non-significant in FSG and Atherogenic because this study excluded any diseases such as metabolic disorder and diabetic. Also in the present study, HO-1level increased in obese groups G3, G4 compared with healthy groups G1, G2, because higher obesity the level of HO-1 increase become more active. These results are shown in Table 3. As a regulator of cellular and tissue homeostasis, a modulator of immunological response and host defense, and inflammation, heme oxygenase-1 (HO-1), a stress protein and metabolic enzyme, continue to pique interest in fundamental and translational investigation on a world-wide scale[19]. These result agree with Abraham N et al.[20]. that HO-1 is related to an increase in cellular heme as well as inflammatory diseases such as atherosclerosis, hypertension, and stroke. The development of potential treatment approaches to reverse the clinical consequences of obesity places a significant emphasis on HO-1. The results also agree with Tirado R, *et al*. [21]. who said that heme oxygenase (HO-1) is an anti-inflammatory enzyme that may become more active in morbid obesity, an enzyme with anti-inflammatory characteristics, heme oxygenase (HO-1) may be elevated in morbid obesity[21]. The results are also in agreement with McClung J *et al*, [22], who found that HO-1 also exerts positive effect by decomposing peroxiding heme, which is higher in obesity. The HO-1 antiadipogenic action is also mediated via CO, BV, and Fe^{+2}. Unfortunately, increasing ROS generation does not induce endogenous HO-1 expression, and as a result, obesity progresses unhindered. Therefore, increasing ROS generation causes HO-1 to be down regulated, which raises the likelihood of developing the metabolic syndrome caused by obesity. By inducing HO-1, a number of pharmaceutical substances that are currently employed in human or animal clinical trials can reduce inflammation. When inflammation starts, increasing HO-1 has no helpful anti-inflammatory benefits, but it has protective effects in myeloid and endothelial cells before inflammation begins. HO positively correlated with G2, G3, and G4 that refers to the relation between HO-1 and obesity by increasing HO-1 secretion when BMI increases that may protect body from problems related to fat, therefor HO-1was positively correlated with Triglyceride levels. The results are in agreement with Ranasinghe, C *et al*. [23], who discovered that in this subpopulation of South Asian people, BMI highly correlated with body fat as measured by bioelectrical impedance. This association was curvilinear in form and was considerably impacted by age. The results showed that age and gender should be taken into account when using BMI to estimate body fat percentage or obesity in a population. This results agree with study by Teller S *et al*. [24], that showed that triglyceride levels are poor predictor of body fat in the current investigation since they had a positive correlation with BMI and body fat.

Conclusion

We concluded from this study that the body's first line of defense against oxidative stress attack is HO-1. This cell-protective enzyme reduces oxidative stress and is vital for controlling lipogenesis, which is crucial for the development of metabolic diseases and its complications.

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Authors' Declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images, that are not ours, have been included with the necessary permission for re-publication, which is attached to the manuscript.
- The author has signed an animal welfare statement.
- Authors sign on ethical consideration's approval.
- Ethical Clearance: The project was approved by the local ethical committee at University of Baghdad. A model oral consent was obtained for all the subject participating in this study. (No:15767, Date 2022/10/4).

Authors' Contribution Statement

N.A.M. performed the acquisition of data analysis, interpretation and drafting the manuscript while F.M.K. did the analysis, design of interpretation, revision and proof reading of the manuscript.

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دراسة دور الهيم اوكسجينيز-1 في المرضى الذين يعانون من السمنة

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قسم الكيمياء، كلية العلوم للبنات، جامعة بغداد، بغداد ،العراق.

الخلاصة

الهيم اوكسجينيز-1 هو عبارة إنزيم محفز يعمل على تكسر جزيئات الهيم إلى بيليفيردين، وحديدالحر، وبيليروبين، وأول أكسيد الكربون. تهدف هذه الدراسة إلى معرفة مستويات ودور (HO-1) في مرضى السمنة ضد الإجهاد التأكسدي وعلاقته بالسمنة. تضمنت الدراسة 139 عينة تضم (84بدناء و55 اشخاص غير بدناء). تم تقسيم كلا مجاميع الدراسة قسمت إلى أربع مجاميع بالاعتماد على مؤشر كتلة الجسم. تم جمع عينات الدم اشخاص بدناء ومجاميع صحية (رجال ونساء) في مستشفى اليرموك والمركز الصحي لأبحاث السكري في الفترة بين ديسمبر2022/ الى يونيو/2023. تم قياس بعض المتغيرات الكيميائية الحيوية لجميع مجموعات الدراسة، والتي تشمل: - تحديد مستويات HO-1 في المصل باستخدام تقنية ELISAو ملف الدهون والسكر الصائم FSG بالطريقة الانزيمية. حيث وجد أن مستويات مؤشر كتلة الجسم تزداد بشكل ملحوظ في مجموعة السمنة من النوع الثاني ومجموعة السمنة من النوع الأول ومجموعة الوزن الزائد مقارنة بمجاميع وزن الطبيعي. كما أظهرت النتائج زيادة معنوية ($p\leq 0.05$)في محيط الخصر الى الورك (WHR) في مجاميع السمنة من النوع الثاني والسمنة من النوع الأول ومجموعة الوزن الزائد مقارنة بمجوعة الوزن الطبيعي. كذلك أظهرت النتائج أن مستويات HO-1 عالية في مرضى السمنة من النوع الاول ومجموعة السمنة من النوع الثاني مقارنةً مع مجاميع الوزن الطبيعي الوزن الزائد. اظهر التحليل الاحصائي أن مستوىHO-1 ترتبط سلبيا مع مؤشر كتلة الجسم في مجموعة الوزن الطبيعي (G1)، بينما إيجابية مع مجاميع السمنة من النوع الثاني والسمنة من النوع الأول والوزن الزائد. نستنتج من هذه الدراسة أن خط دفاع الجسم الأول ضد هجوم الإجهاد التأكسدي هو HO-1. هذا الإنزيم الواقي للخلايا يقلل من الإجهاد التأكسدي وهو حيوي للتحكم في تكون الدهون، وهو أمر حاسم لتطور أمراض التمثيل الغذائي ومضاعفاتها.

الكلمات المفتاحية: مؤشر كتلة الجسم، سكر الصائم، الهيم اوكسجينيز-1، السمنة، نسبة محيط الخصر الى الورك.