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Heme Oxygenase-1 as Crucial Biomarker for Detecting Oxidative Stress and Some Parameters in a Sample of Obese Patients with and without Diabetes

ABSTRACT

Objective: Obesity is a significant contributor to various metabolic disorders, including type 2 diabetes (T2D), resulting in heightened oxidative stress. This study aims to examine the levels of heme oxygenase-1 (HO-1), their correlation with high-density lipoprotein (HDL) concentration, and their influence on the onset of T2D in obese individuals with diabetes.

Materials and methods: The study comprised 150 samples categorized into 3 groups, with each group further subdivided into 2 subgroups: males and females aged 30 to 65 years. Samples were collected at AL-Kindy Teaching Hospital. All sample variables for fasting subjects in every group were assessed. The colorimetric approach was employed for biochemical assays, encompassing fasting blood sugar (FBS) and lipid profiles. Insulin, HO-1, and dipeptidyl peptidase-4 (DPP-4) levels were also quantified using ELISA. Subsequently, we employed statistical analysis to elucidate the results.

Results: Compared to the obesity group, the HO-1 and HDL concentrations were higher in T2D with obesity [(18.0 ± 0.40), (39.86 ± 1.26) vs. (10.41 ± 0.74), (36.27 ± 0.85), with (ng/mL), (mg/dL), respectively]. The T2D with obesity group also showed higher insulin resistance compared to the obesity and control groups [(4.19 ± 0.874) vs. (1.21 ± 0.39), (0.74 ± 0.142)]. The diabetes with obesity male group had elevated HO-1 concentrations compared with the obesity male group, a result that also applied to females.

Conclusions: The T2D with obesity group had higher concentrations of the HO-1 enzyme than the obesity group. We found a positive association between higher HDL concentrations and increased enzyme concentrations in the T2D with obesity group. This enzyme may serve as a biomarker to predict the development of diabetes or the onset of other metabolic diseases. (Clin Diabetol 2024; 13, 6: 349–357)

Keywords: obesity, type 2 diabetes, heme oxygenase-1, dipeptidyl peptidase-4, oxidative stress

Introduction

It is well-acknowledged that obesity is a serious public health issue. Obesity raises the risk of heart disease, metabolic disorders, and some kinds of cancer. Obesity can harm pancreatic islet cells through chronic inflammation, in addition to causing metabolic abnormalities like insulin resistance (IR) and hyperglycemia [1]. Body mass index (BMI), defined as body mass divided by the square of body height and expressed in kilograms per square meter (kg/m^2), is used to deter-

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mine obesity. It is divided into the following groups: (18.5–24.9) normal weight, (25–29.9) overweight, (30–34.9) obesity grade 1, (35–39.9) obesity grade 2, and ≥ 40 extremely severe obesity [2].

Diabetes is a metabolic disease characterized by impaired insulin action, insufficient insulin secretion, or both. It impairs protein, fat, and carbohydrate metabolism [3]. Higher concentrations of hormones, pro-inflammatory cytokines, glycerol, and non-esterified fatty acids are released by obese patients, which may contribute to the development of insulin resistance released by adipose tissue [4]. Insulin is a polypeptide hormone primarily secreted by β cells in the pancreas, namely in islets of Langerhans. To control blood glucose levels, the hormone works in tandem with glucagon; glucagon has catabolic effects, whereas insulin acts through anabolic pathways [5].

One of the numerous hereditary and environmental risk factors linked to obesity and type 2 diabetes (T2D) is oxidative stress. The body's ability to detoxify and generate reactive oxygen species (ROS) can be out of balance, leading to oxidative stress. Because obesity can cause oxidative stress and inflammation through a variety of cellular and metabolic pathways, there is a close relationship between obesity and oxidative stress [6]. Insulin signaling pathways and pancreatic beta cells are negatively impacted by oxidative stress in T2D, accelerating the disease's progression [7].

Heme oxygenase-1 (HO-1) is thought to be the only enzyme that enables cells to break down heme, a molecule that is a component of hemoglobin and other hemoproteins [8]. HO activity produces biliverdin (BV), ferrous iron (Fe2+), and carbon monoxide (CO), which can be made by cleaving heme. Biliverdin reductase (BVR) converts BV to bilirubin (BR) [9]. According to earlier research, by releasing these numerous molecules with anti-inflammatory and antioxidant qualities, HO-1 shields different tissues and organs from oxidative stress and heightened inflammatory reactions [10]. In humans, the absence of HO-1, also referred to as heat shock protein 32 (Hsp32), is linked to anemia among abnormalities in coagulation, early death, growth retardation, and increased iron deposition [8].

Dipeptidyl peptidase-4 (DPP-4) is an enzyme that controls the metabolism of glucose, raising blood sugar levels in people with diabetes through the degradation of incretin hormones, including gastric inhibitory peptide (GIP) and glucagon-like peptide 1 (GLP-1). Upon consumption, various hormones generated from the stomach are produced [11, 12]. This study seeks to ascertain the concentration of the HO-1 enzyme, its correlation with high-density lipoproteins (HDL), and its impact on the progression of diabetes in obese individuals with T2D.

Materials and methods

Collecting, selecting, and analyzing samples

Samples were collected from AL-Kindy Teaching Hospital, and the study was carried out at the College of Science for Women, University of Baghdad, from September 2023 to December 2023. The study included 150 individuals with an age range of 30–65 years. The samples were divided as follows: The first group included 50 samples represented by the control group (healthy individuals) — 25 samples were taken from females and 25 from males. The second group included 50 samples represented by diabetes mellitus (DM) with obesity — 24 samples taken from males, and 26 samples taken from females. The third group included 50 samples, represented by the obesity group — 25 samples taken from females and 25 from males. Following a fasting period of 8 to 12 hours, a 7-mL blood sample was obtained from each participant. The samples were subsequently divided into 4 sections for necessary analyses, which encompassed quantifying the enzyme levels of HO-1, DPP-4, and insulin via ELISA (Cloud-Clone Corp.), as well as conducting additional biochemical assessments, including fasting blood glucose and lipid profile evaluations using linear reagents (S.L.U.). Furthermore, the following formula was employed to ascertain insulin resistance: The Homeostasis Model Assessment of Insulin Resistance (HOMA IR) was calculated as glucose multiplied by insulin divided by 405 (with glucose measured in mg/dL). Additionally, the BMI was determined using the formula: weight divided by height squared, and the waist-to-hip ratio (WHR) was assessed with a tape measure.

Exclusion criteria

This study excluded the following individuals: thyroid patients and those with kidney disease, individuals suffering from heart disease, pregnant women, and patients using insulin injections to treat diabetes and diabetic complications.

Statistical analysis

All statistical analyses were conducted using version 26.0 of the SPSS program. Pairwise post hoc comparisons were employed among many groups, alongside variance analysis (ANOVA), ROC curve analysis, and the correlation coefficient (r) between parameters. The data was exhibited using a normal distribution represented as mean ± standard error (SE). A p-value

Table 1. Mean ± SE between patient and control groups with all parameters

Groups Parameters	Control group (N = 50)	Diabetes mellitus with obesity group (N = 50)	Obesity group (N = 50)	P-value
Age [year]	38.30 ± 1.25 [a]	51.96 ± 1.15 [c]	44.20 ± 1.43 [b]	0.001**
BMI [kg/m^2]	23.35 ± 0.28 [a]	33.52 ± 0.41 [b]	34.49 ± 0.615 [b]	0.001**
W/H ratio	0.96 ± 0.031 [a]	1.00 ± 0.014 [a]	0.95 ± 0.018 [a]	0.310
FBS [mg/dL]	96.87 ± 1.01 [a]	194.24 ± 10.54 [b]	100.74 ± 1.18 [a]	0.001**
Insulin [μIU /mL]	3.05 ± 0.584 [a]	8.06 ± 1.39 [b]	4.80 ± 1.54 [ab]	0.018*
HOMA IR	0.74 ± 0.142 [a]	4.19 ± 0.874 [b]	1.21 ± 0.39 [a]	0.001**
TC [mg/dL]	174.54 ± 2.75 [a]	189.26 ± 7.68 [a]	179.24 ± 3.83 [a]	0.128
TG [mg/dL]	128.28 ± 3.72 [a]	216.56 ± 19.82 [b]	136.56 ± 6.06 [a]	0.001**
HDL-C [mg/dL]	42.42 ± 0.542 [b]	39.86 ± 1.26 [b]	36.27 ± 0.85 [a]	0.001**
LDL-C [mg/dL]	106.46 ± 2.70 [a]	109.26 ± 7.0 [a]	115.66 ± 3.34 [a]	0.376
VLDL-C [mg/dL]	25.66 ± 0.74 [a]	45.45 ± 4.39 [b]	27.31 ± 1.21 [a]	0.001**
HO-1 [ng/mL]	15.20 ± 0.43 [b]	18.0 ± 0.40 [c]	10.41 ± 0.74 [a]	0.001**
DPP-4 [ng/mL]	15.55 ± 0.83 [b]	7.71 ± 0.28 [a]	6.48 ± 0.27 [a]	0.001**

**Significant difference between means using ANOVA -test at 0.01 level

BMI — body mass index; DPP-4 — dipeptidyl peptidase-4; FBS — fasting blood sugar; HDL-C — high-density lipoprotein cholesterol; HO-1 — heme oxygenase-1; HOMA IR — Homeostasis Model Assessment of Insulin Resistance; LDL-C — low-density lipoprotein cholesterol; SE — standard error; TC — total cholesterol; TG — triglycerides; VLDL-C — very low-density lipoprotein cholesterol; WHR — waist-to-hip ratio

≤ 0.05 indicated a significant difference, considered a statistical signal.

Results

The results of age, BMI, fasting blood sugar (FBS), insulin, HOMA IR, triglycerides (TG), HDL cholesterol, very low-density lipoprotein cholesterol (VLDL-C), HO-1, and DPP-4 were significant ($p \leq 0.05$) between groups. The age showed a mean ± SE of 51.96 ± 1.15 years for DM with obesity and 44.20 ± 1.43 years for obesity, a high value compared with the control group, at 38.30 ± 1.25 years. The patient group showed a high BMI mean value for DM with obesity compared with the control group. As in Table 1, for FBS and HOMA IR for DM with obesity compared with obesity and control groups [(194.24 ± 10.54 mg/dL), (4.19 ± 0.874 mg/dL) vs. (100.74 ± 1.18 mg/dL) (1.21 ± 0.39 mg/dL), (96.87 ± ± 1.01 mg/dL) (0.74 ± 0.142 mg/dL)]. The statistical function showed a significant difference in the probability value of insulin in the DM with obesity group compared to the control group.

The results showed that there was a statistically significant increase in the p-value in triglycerides, HDL cholesterol, and (VLDL-C); the mean value ± SE increased significantly between groups, but there was no significant difference for total cholesterol and low-density lipoproteins (LDL). As in Table 1, the patients' group showed a mean value of triglycerides ± SE of 216.56 ± 19.82 mg/dL for DM with obesity, a high value compared to 136.56 ± 6.06 mg/dL for obesity, and the control group at 128.28 ± 3.72 mg/dL. While the HDL value was lower in obesity (36.27 ± 0.85 mg/dL) compared to DM with obesity (39.86 ± 1.26 mg/dL) and the control group (42.42 ± 0.542 mg/dL), the mean value of VLDL increased for DM with obesity compared to the obesity group and the control group. The mean value between groups increased significantly in HO-1 and DPP-4.

The patient groups showed an increase in the mean heme oxygenase-1 value ± SE (18.0 ± 0.40 ng/mL) for DM with obesity and a low value (10.41 ± 0.74 ng/dL) for obesity compared with the control group (15.20 ± ± 0.43 ng/dL). The mean value ± SE of DPP-4 decreased for (6.48 ± 0.27 ng/mL) obesity and (7.71 ± ± 0.28 ng/mL) DM with obesity compared to the control group (15.55 ± 0.83 ng/mL), as indicated in Table 1.

Table 2 shows the correlation of different parameter levels with HO-1 in groups. The results showed a positive correlation between HO-1 and BMI, insulin, and HOMA IR in the control group, DM and obesity, and a significantly positive correlation with HDL in DM with obesity. At the same time, there was a negative significant correlation in the control group and the obesity group, while a significant positive and strong correlation was seen between HO-1 and DPP-4 in DM with obesity.

Table 2. Correlations between HO-1 and all parameters

		Heme oxygenase-1 [ng/mL]		
		Control group (N = 50)	Diabetes mellitus with obesity group (N = 50)	Obesity group (N = 50)
Age [years]	r	0.057	–0.063	–0.083
	P	0.692	0.666	0.567
BMI [kg/m^2]	r	0.298*	0.462**	0.115
	P	0.035	0.001	0.426
WHR	r	0.236	–0.073	0.030
	P	0.099	0.615	0.837
FBS [mg/dL]	r	0.007	0.229	0.110
	P	0.963	0.109	0.449
TC [mg/dL]	r	0.200	–0.005	–0.005
	P	0.164	0.971	0.973
TG [mg/dL]	r	0.079	–0.124	0.113
	P	0.584	0.389	0.434
HDL-C [mg/dL]	r	–0.302*	0.289*	–0.300*
	P	0.033	0.041	0.034
LDL-C [mg/dL]	r	0.204	–0.075	0.030
	P	0.155	0.607	0.837
VLDL-C [mg/dL]	r	0.001	–0.160	0.113
	P	0.995	0.267	0.434
Insulin [μlU /mL]	r	0.319*	0.497**	–0.027
	P	0.024	0.000	0.852
HOMA IR	r	0.314*	0.456**	–0.036
	P	0.026	0.001	0.806
DPP-4 [mg/dL]	r	–0.161	0.315*	0.281*
	P	0.265	0.026	0.048

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

BMI — body mass index; DPP-4 — dipeptidyl peptidase-4; FBS — fasting blood sugar; HDL-C — high-density lipoprotein cholesterol; HO-1 — heme oxygenase-1; HOMA IR — Homeostasis Model Assessment of Insulin Resistance; LDL-C — low-density lipoprotein cholesterol; SE — standard error; TC — total cholesterol; TG — triglycerides; VLDL-C — very low-density lipoprotein cholesterol; WHR — waist-to-hip ratio

Alongside our study, which comprised a cohort of patients and healthy controls, a cross-sectional analysis was performed, including male and female patients. The male group with DM and obesity had a mean age ± SE of 53.16 ± 1.78 years, significantly higher than the obesity-only male group, at 43.20 ± 2.09 years, as depicted in Table 3. The average BMI values of the DM with obesity group comprising fat males and obese females exhibited a statistically significant difference. Obesity females exhibited a higher BMI than DM with obesity males, measuring 35.67 ± 0.85 vs. 32.32 ± ± 0.31 kg/m^2. The WHR in DM with obesity males was greater than that in obesity females. Results for FBS, insulin, and HOMA IR between male and female patients indicate that the mean ± SE of FBS in DM with obesity males was higher than that in obesity females. Additionally, the mean ± SE for DM and obesity females was greater than that for obesity females. Insulin and insulin resistance (HOMA IR) exhibited elevated mean ± SE values in DM with obesity females compared to their male counterparts, namely 10.42 ± 2.49 μlU/mL, 5.09 ± 1.50 μlU/mL vs. 1.75 ± 0.51 μlU/mL, 0.43 ± ± 0.12 μlU/mL, respectively.

The mean value ± SE increased significantly ($p \leq 0.05$) between groups concerning triglycerides, HDL, and VLDL, while no group showed a significant value ($p > 0.05$) for LDL and total cholesterol between groups. When comparing males and females in the patient group. The mean triglyceride values ± SE were highest in DM with obesity males compared to obesity males and obesity females. The values were 226.45 ± ± 33.24 mg/dL, 141.84 ± 9.63 mg/dL, and 131.28 ± ± 7.42 mg/dL, respectively. The mean HDL ± SE for females suffering from DM with obesity revealed a high value in comparison with DM with obesity males and obesity female groups (43.34 ± 1.87 vs. 36.08 ± 1.33, 36.54 ± 0.96 mg/dL). The DM with obesity male group showed a high mean value ± SE for VLDL compared with

Table 3. Mean ± SE between males and females in patient groups with all parameters

Groups Parameters	Diabetes mellitus with obesity male group (N = 24)	Diabetes mellitus with obesity female group (N = 26)	Obesity female group (N = 25)	Obesity male group (N = 25)	P-value
Age [year]	53.16 ± 1.78 ^{c}	50.84 ± 1.48 ^{bc}	45.20 ± 1.98 ^{ab}	43.20 ± 2.09 ^{a}	0.001**
BMI [kg/m^2]	32.32 ± 0.31 ^{a}	34.62 ± 0.66 ^{ab}	35.67 ± 0.85 ^{b}	33.31 ± 0.83 ^{ab}	0.007**
WHR	1.03 ± 0.02 ^{b}	0.97 ± 0.01 ^{ab}	0.90 ± 0.009 ^{a}	1.0 ± 0.03 ^{b}	0.001**
FBS [mg/dL]	202.16 ± 18.65 ^{b}	186.92 ± 10.89	100.31 ± 1.66 ^{a}	101.17 ± 1.72 a	0.001**
Insulin [μIU /mL]	5.51 ± 0.90 ^{ab}	10.42 ± 2.49 ^{b}	7.85 ± 2.95 ^{ab}	1.75 ± 0.51 a	0.023*
HOMA IR	3.21 ± 0.80 ^{ab}	5.09 ± 1.50 ^{b}	1.97 ± 0.75 ^{ab}	0.43 ± 0.12 a	0.006**
TC [mg/dL]	182.5 ± 9.92 ^{a}	195.5 ± 11.64 ^{a}	179.80 ± 5.86 ^{a}	178.68 ± 5.06 ^{a}	0.480
TG [mg/dL]	226.45 ± 33.24 ^{b}	207.42 ± 23.13 ^{ab}	131.28 ± 7.42 ^{a}	141.84 ± 9.63 ^{a}	0.003**
HDL-C [mg/dL]	36.08 ± 1.33 ^{a}	43.34 ± 1.87 ^{b}	36.54 ± 0.96 ^{a}	36.0 ± 1.42 ^{a}	0.001**
LDL-C [mg/dL]	104.29 ± 8.85 ^{a}	113.84 ± 10.79 ^{a}	117.0 ± 5.11 ^{a}	114.31 ± 4.40 ^{a}	0.689
VLDL-C [mg/dL]	46.19 ± 6.84 ^{c}	44.76 ± 5.73 ^{bc}	26.25 ± 1.48 ^{a}	28.36 ± 1.92 ^{ab}	0.002**
HO-1 [ng/mL]	16.90 ± 0.62 ^{b}	19.0 ± 0.46 ^{b}	11.53 ± 0.84 ^{a}	9.29 ± 1.19 ^{a}	0.001**
DPP-4 [ng/mL]	7.10 ± 0.38 ^{ab}	8.26 ± 0.40 ^{b}	6.53 ± 0.34 ^{a}	6.43 ± 0.44 ^{a}	0.004**

**Significant difference between means using ANOVA -test at 0.01 level

BMI — body mass index; DPP-4 — dipeptidyl peptidase-4; FBS — fasting blood sugar; HDL-C — high-density lipoprotein cholesterol; HO-1 — heme oxygenase-1; HOMA IR — Homeostasis Model Assessment of Insulin Resistance; LDL-C — low-density lipoprotein cholesterol; SE — standard error; TC — total cholesterol; TG — triglycerides; VLDL-C — very low-density lipoprotein cholesterol; WHR — waist-to-hip ratio

Table 4. ROC curve analysis of HO-1 between obesity and diabetes mellitus with obesity groups

Area under the curve					
Test result variable(s)	Area	Std. error[a]	Asymptotic Sig.[b]	Asymptotic 95% confidence interval	
				Lower bound	Upper bound
Heme oxygenase-1 (ng/mL)	.886	.036	.000	.816	.956

HO-1 — heme oxygenase-1

[a]Under the nonparametric assumption; [b]Null hypothesis: true area = 0.5

the obesity male group. A high value for DM with obesity females was seen compared with the obesity male group.

Table 3 shows that the mean ± SE values of DM with obesity males, which is high compared with obesity males (16.90 ± 0.62 vs. 9.29 ± 1.19 ng/mL), and the mean for DM with obesity females is high compared to obesity females (19.0 ± 0.46 vs. 11.53 ± 0.84 ng/mL). The mean value increased for DPP-4 in DM with obesity females compared with obesity females and males (8.26 ± 0.40 vs. 6.53 ± 0.34 and 6.43 ± 0.44 ng/mL).

Table 4 presents the findings of the ROC analysis using the following parameters between the groups of obesity and DM with obesity. The area under the ROC curve (AUC) for OH-1 demonstrated good diagnostic accuracy with a value of 0.886. The cut-off value for OH-1 (16.30) and the sensitivity and specificity of HO-1 (0.70 and 0.16, respectively) are shown in Figure 1.

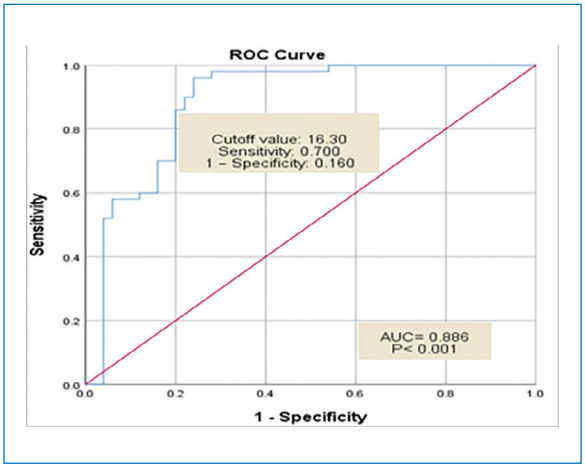

Figure 1. ROC curve analysis of HO-1 for obesity and diabetes mellitus with obesity group
AUC — area under the curve; HO-1 — heme oxygenase-1

Discussion

Table 1 displays the results, revealing a statistically significant difference in BMI between the control and patient groups. The correlation between a higher body mass index and an increased risk of T2D is evident [13]. Obese patients who accumulate significant amounts of body fat have a higher likelihood of developing T2D because obesity influences both insulin action and B-cell function. This finding aligns with the research conducted by Klein et al. [14]. Research indicates that males exhibit a higher susceptibility to T2D compared to females, with diagnoses occurring at lower BMI levels in males than in females. The current study indicates, as illustrated in Table 3, that males with DM and obesity exhibit a lower BMI than females. Furthermore, it has been noted that men typically exhibit a greater tendency to accumulate weight in the abdominal area, whereas women are more inclined to store weight in the hips and thighs. There is a correlation between abdominal fat and an elevated risk of developing diabetes. The findings align with the research presented in study [15]. Furthermore, individuals with a high BMI exhibit a greater propensity for developing T2D, with women showing a higher likelihood than men. The results are consistent with the findings of previous research [16]. Furthermore, males exhibiting a higher waist-to-hip ratio demonstrate a greater vulnerability to insulin resistance and various metabolic irregularities compared to females. The results of our analysis corresponded with those of a previous study [17]. Our study's results indicate a statistically significant difference between the patient group and the healthy group, highlighting a notable association between the increasing risk of developing diabetes and advancing age. The probability of an individual developing heart disease generally increases as they age [18].

The results presented in Table 1 indicate that the average values of FBS, INS, and HOMA-IR show a significant upward trend in the DM with obesity group compared to both the control group and the obesity group ($p \leq 0.05$). The findings align with the research conducted by Abed et al. [19]. Obesity represents a significant risk factor for diabetes, closely linked to the phenomenon of insulin resistance. The adipose tissue in obese individuals secretes elevated levels of hormones, pro-inflammatory cytokines, glycerol, and non-esterified fatty acids, potentially playing a role in the onset of insulin resistance. Additionally, oxidative stress and lipodystrophy impact insulin resistance, as demonstrated in the research conducted by Wondmkun et al. [20]. Table 3 presents results showing that females experienced a greater increase in HOMA IR value compared to males. The study's findings are consistent with earlier research [21]. Although various research findings contradict our own, which indicate that men are more prone to developing obesity, insulin resistance, and hyperglycemia in response to nutritional challenges, it is evident that women exhibit distinct energy partitioning patterns in comparison to men. Fat and carbohydrates serve as fuel sources, facilitating energy storage in subcutaneous adipose tissues while safeguarding against visceral and ectopic fat accumulation. Women exhibit a greater insulin sensitivity than men do [22].

A previous study showed that DM with obesity has higher triglyceride levels than those with obesity, as indicated in a study conducted by Aljabri et al. [23], which was consistent with our current study, as displayed in Table 1. Individuals suffering from DM with obesity have higher levels of triglyceride deposition in non-adipose tissue. A decrease in HDL cholesterol was observed. Fat accumulation in the visceral and abdominal subcutaneous depots is strongly associated with the risk of metabolic and cardiovascular issues. The results are consistent with the study by Khalid Jaid et al. [24]. Advanced end products of inflammation, oxidative stress, and hyperglycemia induce dysregulation of HDL cholesterol in diabetes. This elevates the risk of cardiovascular disease. Our findings align with those of Abed et al. [25]. Table 3 indicates that triglyceride levels were elevated in obese males with DM compared to obese females, although HDL levels were greater in obese females than in males. Consequently, the findings of our research align with those of a prior study [23]. A greater amount of HDL further substantiates the advantageous benefits of estrogen.

The present research revealed that serum HO-1 concentrations were markedly elevated in individuals with DM accompanied by obesity compared to the control group. The findings of our research align with those presented by Bao et al. [26], which indicated a heightened level of HO-1 in the plasma of individuals diagnosed with T2D. The observed increase is thought to correlate with heightened oxidative stress in these individuals, stemming from the generation of significant quantities of free radicals capable of inflicting cellular damage. The elevation of the HO-1 enzyme is viewed as a component of the body's protective mechanism against oxidative stress and is crucial in mitigating disease complications.

Individuals with T2D and obesity demonstrate elevated HO-1 activity, which correlates with increased levels of plasma glucose, iron, and thiobarbituric acid reactive substances (TBARS), suggesting a potential rise in stress levels [27, 28]. Obese patients exhib-

ited reduced levels of HO-1 when compared to the healthy group. The increase in ROS production leads to a reduction in HO-1 levels. This increases the likelihood of developing metabolic syndrome associated with obesity [29]. In this study, it was observed that diabetic males with obesity exhibited elevated levels of the enzyme HO-1 compared to their non-diabetic obese counterparts, and a similar pattern was noted among females. HO-1 serves as the body's primary line of defense against oxidative stress. This enzyme plays a crucial role in the regulation of adipogenesis, a process that is significant in the development of obesity and contributes to the reduction of oxidative stress. Our findings indicate a correlation between obesity and metabolic syndrome in obese females and the presence of inflammation, which subsequently elevates reactive oxygen species (ROS) levels. The oxidant assault increases isoprostane levels and leads to the oxidation of HDL (Ox-HDL) [15]. Table 2 illustrates a correlation between HO-1 enzyme concentration and HDL levels in the context of DM accompanied by obesity. Men experiencing metabolic syndrome and aging demonstrate elevated rates of morbidity and mortality. Lower levels of stress proteins, particularly intracellular HO-1, contribute to their increased susceptibility to illness [30]. Numerous studies utilizing animal models and data from individuals with insufficient HO-1 indicate its significant role in various clinical scenarios characterized by elevated inflammation and oxidative stress levels. Pharmacological therapy aimed at stimulating HO-1 production represents a novel and promising strategy for the management of inflammatory diseases [16].

Research suggests that DM accompanied by obesity may have elevated DPP-4 levels. Elevated DPP-4 levels may diminish the efficacy of incretins, resulting in reduced insulin secretion and glucose intolerance [31]. This contrasts with our findings: Vildagliptin, an oral medication for T2D, reduced DPP-4 levels. These pharmaceuticals are referred to as DPP-4 inhibitors, functioning by inhibiting the enzyme DPP-4 from degrading incretin hormones such as glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). Consequently, these drugs elevate GIP and GLP-1 levels, enhancing glucose control by augmenting insulin secretion and diminishing glucagon secretion [32]. Studies have shown that DPP-4 inhibitors protect cells against several diabetes-related problems affecting the kidneys, liver, heart, retina, and neurons [33]. In comparison to obese females and obese males, the cohort of DM with obese females had a higher mean value of DPP-4. The conclusions of the investigation correspond with those of a prior study [34].

Table 4 indicates that the ROC analysis of HO-1 produced favorable outcomes. This suggests that this enzyme could serve as a biomarker for predicting the progression of diabetes or the emergence of other metabolic disorders.

Conclusions

DM associated with obesity demonstrated a higher concentration of the HO-1 enzyme in comparison to the obesity group alone. Male subjects with DM and obesity exhibited higher levels of the HO-1 enzyme compared to their male counterparts who had obesity without diabetes. Obese females with DM exhibited higher enzyme concentrations compared to their non-diabetic counterparts. This increase can be linked to the enzyme's antioxidant and anti-inflammatory properties, which potentially reduces the risk of T2D and other metabolic disorders. A positive correlation was identified with HDL, indicating that higher HDL levels were associated with increased enzyme concentrations in individuals with DM in the obese group. This enzyme could serve as a potential indicator for predicting the advancement of diabetes or the onset of various metabolic disorders.

Article information

Data availability statement

All research data are accessible on reasonable inquiry.

Ethics statement

The Declaration of Helsinki's ethical guidelines guided the research. We conducted the procedure after obtaining the patients' verbal and analytical consent prior to sample collection. The research protocol, subject information, and permission form underwent assessment and approval by the local Ethical Committee at the University of Baghdad.

Author contributions

Mays Mohammed Abdullah was responsible for collecting samples, conducting analysis, interpreting data, writing the manuscript, and proofreading it. Fayhaa Muqdad Khaleel conceived the idea, supervised the research, and read the manuscript.

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Conflict of interest

The authors declare no conflict of interest.

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