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EVALUATION OF OXIDATIVE STRESS, ANTIOXIDANT DEFENSES, AND BIOCHEMICAL DYSREGULATION IN OBESE VS. NON-OBESE ADULT MALES

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ABSTRACT:

Obesity is a major public health concern linked to metabolic disturbances and increased oxidative stress. The objective of this research is to evaluate the effect of obesity on oxidative–antioxidant balance in adult males. Comparative cross-sectional study was done at Zakho General Hospital, Iraq, from October 2024 to January 2025, involving 90 males aged 18–44 years, distributed into obese body mass index (BMI) ≥30 and non-obese body mass index (BMI) <25 groups. Blood samples were collected and analyzed for biochemical, and oxidative stress parameters using Cobas auto-analyzers and spectrophotometric methods. Obese individuals exhibited significantly higher body mass index (BMI), waist circumference (WC), diastolic blood pressure (DBP), fasting blood glucose (FBG), fasting insulin (FI), lipid profile, Liver enzymes (aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT)), Kidney function parameters (urea, creatinine and uric acid), High-sensitivity C-reactive protein (hs-CRP) and homeostatic model assessment of insulin resistance (HOMA-IR) in comparison with controls. Concentrations of Zinc (Zn) and copper (Cu) were elevated, whereas magnesium (Mg) was decreased in the obese group. Antioxidant markers (glutathione S-transferase (GST), superoxide dismutase (SOD), and catalase) were significantly reduced in obese group. Strong negative correlations are observed between oxidative markers and most anthropometric and biochemical parameters. Obesity in adult males is associated with impaired antioxidant defense and alterations in trace elements, emphasizing the oxidative stress burden in obese individuals and the need for early preventive strategies.

KEYWORDS: Obesity, Oxidative Stress, Antioxidants, Trace Elements, Metabolic Parameters

1. INTRODUCTION

Obesity is a chronic disease characterized by excessive fat accumulation that could impair health(WHO, 2024). The World Health Organization (WHO) recognizes the obesity as a global epidemic, it affects individuals of all ages and socioeconomic backgrounds. As of 2022, over 890 million adults worldwide were obese, with numbers projected to rise in the coming years. Obesity is linked to serious health conditions, such as type 2 diabetes, cardiovascular disease, hypertension, non-alcoholic fatty liver disease, and specific cancers(Kirichenko *et al.*, 2022; WHO, 2024).

One of the critical pathophysiological mechanisms contributing to the development of obesity-related complications is oxidative stress (Čolak & Pap, 2021). The imbalance between the reactive oxygen species (ROS) production and the ability of the body to neutralize them through antioxidant defenses is known as oxidative stress (Hami & Yousif, 2025 ; Ji & Yeo, 2021) . In normal physiological state, hydrogen peroxide (H_2O_2), superoxide anion (O_2^-), and hydroxyl radicals (OH•) are essential for normal cellular signaling and immune defense. The overproduction of these ROS leads to the damage of lipids, proteins and DNA, resulting in cellular dysfunction, inflammation, and programmed cell death (Sachdev *et al.*, 2021; Sadiq, 2023). In obesity, excessive adipose tissue becomes a site of increased oxidative metabolism and chronic inflammation, both enhance ROS production and reduce antioxidant capacity (Yu *et al.*, 2023).

The human antioxidant defense systems comprises both enzymatic and non-enzymatic systems, such as catalase, glutathione peroxidases (GPx), superoxide dismutase (SODs), glutathione S-transferases (GST), glutathione reductase (GR), vitamins C, E, carotenoids, flavonoids, uric acid, bilirubin, and glutathione which collectively act to neutralize reactive oxygen species (ROS) and repair molecules damaged by oxidative stress (Janciauskiene, 2020; Irato & Santovito, 2021; Jomova *et al.*, 2024; Vilchis-Landeros *et al.*, 2024)

Trace elements play a vital role in maintaining oxidative balance. These essential micronutrients serve as cofactors for many enzyme-catalyzed reactions and redox reactions (Wróblewski *et al.*, 2024). Zinc (Zn) and copper (Cu) are essential for the integrity and catalytic activity of Cu/Zn-SOD (Székely *et al.*, 2024). Manganese (Mn) is an essential cofactor for Mn-SOD,

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enabling its antioxidant activity in mitochondria. Magnesium (Mg) is involved in ATP production and stabilizes antioxidant enzymes, while chromium (Cr) and molybdenum (Mo) contribute to maintaining redox homeostasis (Jomova *et al.*, 2022). Trace element imbalances, whether from deficiency or excess, can disrupt antioxidant enzyme function and facilitating oxidative stress, particularly in obesity (Amin *et al.*, 2020).

Although the role of oxidative stress and trace element imbalance in obesity has been well studied in many parts of the world, data from specific populations remain limited (Jakubiak *et al.*, 2021; Amerikanou *et al.*, 2023). Local variations in dietary habits, genetic predispositions, and environmental exposure may influence these biochemical interactions(Almeida & Barakat, 2021).

This study aims to evaluate the oxidative–antioxidant balance and trace element status in obese versus non-obese adult males in Zakho City, and to assess the association of these antioxidant markers with various anthropometric and biochemical parameters.

2. MATERIALS AND METHODS

Design of the Study:

This comparative cross-sectional study was carried out over four months, from October 2024 to January 2025, at Zakho General Hospital in Zakho City, Kurdistan Region, Iraq. Ninety adult males aged between 18 and 44 were recruited for the investigation. Prior enrollment, adult males were informed about the objective of this study and written consent was singed from each participant. Basic demographic and health information, such as age, location, lifestyle behaviors, and medical history, was obtained through a questionnaire developed for this research.

These individuals were divided into two groups depending on their body mass index (BMI): Group 1 comprised 50 individuals classified as obese; BMI ≥ 30 kg/m², while Group 2, considered as control group, included 40 participants; BMI between 18.5 and 24.99 kg/m². Individuals were excluded from participation if they had any diagnosed chronic illnesses such as diabetes mellitus, thyroid disorders, or cardiovascular disease, or if they were taking medications, drinking alcohol, or using tobacco in any form.

Anthropometric measurements were recorded for all participants. These measurements included: body weight and height for calculating BMI, and waist circumference (WC). In addition, systolic (SBP) and diastolic blood pressure (DBP) readings were measured for all adult males.

Six mL of venous blood was drawn from each participant between 8:30 a.m. to 12:00 p.m., following a minimum of 8 hours of overnight fasting and then transferred into gel-based serum separation tubes. After that, the samples are allowed to clot at room temperature, then centrifuged at 3000 rpm for 10 minutes using a Universal 320 centrifuge (Hettich, Germany). The resulting serum was transferred into pre-labeled 1.0 mL Eppendorf tubes and stored at –75 °C in deep freezers at the Zakho Blood Bank laboratory until further biochemical analyses were performed.

Biochemical Analysis:

The following biochemical parameters; fasting blood glucose (FBG), urea, creatinine, uric acid, lipid profile, liver function tests (alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP); and gamma-glutamyl transferase (GGT), and high-sensitivity C-reactive protein (hs-CRP) were measured using an automated Cobas 6000 c501 chemistry analyzer (Roche Diagnostics, HITACHI). Fasting insulin (FI) levels are analyzed using the Cobas 6000 c601 immunoassay system (Roche Diagnostics). Some parameters, which include very low-density lipoprotein cholesterol (VLDL-C), low-density lipoprotein cholesterol (LDL-C), and Homeostatic Model Assessment of Insulin Resistance (HOMA-IR), were estimated by using these equations (VLDL-C = TG/5), (LDL-C = TC − HDL-C − TG/5)(Friedewald *et al.*, 1972), and (HOMA-IR) = FBS (mg/dL) * FI (mU/ml)/405(Pourhabibi-Zarandi *et al.*, 2022).

The enzymatic activities of glutathione S-transferase (GST), superoxide dismutase (SOD), and catalase (CAT) in human serum were determined using spectrophotometric methods. All absorbance readings were measured using a UV/VIS spectrophotometer (Perkin Elmer Lambda 35, USA).

GST and SOD activities were determined using the methods described by (Ibrahiem *et al.*, 2024). GST assay is based on the conjugation of reduced glutathione (GSH) with 1-chloro-2,4-dinitrobenzene (CDNB), catalyzed by GST to form a GS-DNB complex that absorbs light at 340 nm. The reaction mixture contained 100 µL of serum, 100 µL of CDNB (2.5 mM), 100 µL of GSH (5 mM), and 700 µL of potassium phosphate buffer (0.1 M, pH 5.5). A blank was prepared using buffer instead of serum. After incubation at 37 °C for 6 minutes, the absorbance was measured at 340 nm. The enzymatic activity was calculated based on the Beer–Lambert Law with the extinction coefficient ε = 9.6 mM^{-1} cm^{-1}.

Superoxide dismutase (SOD) activity was determined using the photochemical nitroblue tetrazolium (NBT) method. The assay principle is based on the ability of SOD to inhibit the photoreduction of NBT by superoxide radicals generated in a riboflavin–methionine–light system. The assay mixture contained 3 mL of reaction solution prepared as follows: 117 mL of phosphate buffer (50 mM, pH 7.8), 1.25 mL of L-methionine (0.2 M), 1 mL of NBT (1.75 mM), and 0.75 mL of Triton X-100 (1%). To this, 39 µL of sodium cyanide (2 mM) was added as a peroxidase inhibitor, followed by 150 µL of serum, 532 µL of working buffer (prepared by dissolving 0.0375 g EDTA and 0.25 mL Triton X-100 in 100 mL of 50 mM phosphate buffer, pH 7.8), and 37.8 µL of riboflavin solution (117 µM). A blank was prepared using working buffer in place of serum. After thorough mixing, the samples were placed in a closed wooden box lined with aluminum foil and exposed to intense light from two 20-watt fluorescent lamps for 15 minutes. The absorbance was measured at 560 nm before and after light exposure. The difference in absorbance was used to estimate the SOD activity.

Catalase activity was measured using the method described by (Hadwan & Abed, 2016), which is based on the decomposition of hydrogen peroxide (H_2O_2) by catalase in the presence of a phosphate buffer. The remaining H_2O_2 reacts with ammonium molybdate to form a yellow complex, which is measured spectrophotometrically at 374 nm. The reaction mixture for the test sample consisted of 100 µL of serum and 1000 µL of H_2O_2 (20 mM) in phosphate buffer (50 mM, pH 7.4). After vortexing, the mixture was incubated at 37 °C for 3 minutes. The reaction was then stopped by adding 4000 µL of ammonium molybdate (32.4 mM). For the control-test, H_2O_2 was omitted, while the blank contained only distilled water (1100 µL) and ammonium

molybdate. The standard consisted of H_2O_2 (1000 µL) and buffer (100 µL), followed by molybdate. All samples were kept at room temperature after the addition of molybdate, and absorbance was recorded at 374 nm using a UV/VIS spectrophotometer (Perkin Elmer Lambda 35, USA). Catalase activity was calculated using the first-order reaction rate constant formula:

$$\text{Catalase activity (kU)} = \frac{2.303}{t} * \left[\log\frac{S°}{S-M}\right] \times \frac{Vt}{Vs}$$

t: time. S°: absorbance of standard tube. S: absorbance of test tube. M: absorbance of control test (correction factor). Vt: total volume of reagents in test tube. Vs: volume of serum.

The concentrations of trace elements, including zinc (Zn), copper (Cu), and magnesium (Mg), were determined using commercially available colorimetric reagent kits supplied by Spectrum Diagnostics (Germany) and BioSystems (Spain). All measurements were performed using a JENWAY 6700 visible spectrophotometer (UK).

Serum zinc concentration was quantified using a colorimetric method based on the 5-Bromo-PAPS assay (Spectrum Diagnostics, REF 232001, Germany). This method is based on the formation of a red-colored complex between zinc and2-(5-Bromo-2-pyridylazo)-5-(N-propyl-N-sulfopropylamino)-phenol. The intensity of the color is directly proportional to the concentration of zinc in the sample. The reagent contained 5-Br-PAPS (0.02 mmol/L), bicarbonate buffer (200 mmol/L, pH 9.8), sodium citrate (170 mmol/L), dimethylglyoxime (4 mmol/L), and 1% detergent.

In the assay procedure, 1 ml of the working reagent was mixed with 50 µl of serum or zin standard solution, while the blank contained only the reagent. The mixture was incubated at 37 °C for 5 minutes. Absorbance was measured at 546 nm against a reagent blank. Zinc concentration in µg/dl was calculated using the following formula:

$$\text{Zinc concentration (µg/dl)} = (\text{A\_sample} / \text{A\_standard}) \times 200$$

serum copper concentration was determined using a colorimetric method with Dibromo-PAESA, supplied by Spectrum Diagnostics (Germany; REF 232001). The method is based on the formation of a stable colored chelate complex between copper and 4-(3,5-dibromo-2-pyridylazo)-N-ethylsulfopropylaniline under acidic conditions. The intensity of the complex is directly proportional to the copper concentration in the sample. The reagent composition included acetate buffer (0.2 mol/L, pH 5.0) and Dibromo-PAESA (0.02 mmol/L) as a single monoreagent.

In the assay, 1.0 ml of reagent was added to 50 µl of either the serum sample or copper standard (100 µg/dL), while the blank contained only the reagent. All tubes were thoroughly mixed and incubated at 37 °C for 5 minutes. After incubation, the absorbance of the sample (A_s) and standard (A_{st}) was measured against the reagent blank at 580 nm. The copper concentration (µg/dL) was calculated using the following equation:

$$\text{Copper concentration (µg/dL)} = (\Delta A_s / \Delta A_{st}) \times 100$$

where ΔA represents the difference between sample or standard absorbance and the blank.

Serum magnesium concentration was determined using a colorimetric method based on the reaction with xylidyl blue, supplied by BioSystems (Spain; COD 11797). The method relies on the formation of a colored complex between magnesium and xylidyl blue in an alkaline medium. EGTA is incorporated in the reagent to eliminate interference from calcium. The intensity of the resulting complex is directly proportional to the magnesium concentration in the sample. The working reagent was prepared by combining Reagent B (xylidyl blue 0.5 mmol/L, glycine 25 mmol/L, and chloroacetamide 2.6 g/L) with Reagent A (sodium carbonate 0.1 mol/L, EGTA 0.1 mmol/L, triethanolamine 0.1 mol/L, potassium cyanide 7.7 mmol/L, and sodium azide 0.95 g/L). In the assay, 1.0 mL of the working reagent was added to 10 µL of either the serum sample or the magnesium standard (2 mg/dl). A blank was prepared using only the working reagent. All tubes were mixed thoroughly and incubated at room temperature for 2 minutes. The absorbance of the sample (A_s) and standard (A_{st}) was measured against the reagent blank at 520 nm. The magnesium concentration in the sample was calculated using the following formula:

$$Magnesium\ (mg/dL) = \left(\frac{As}{Ast}\right) \times Cst$$

A_S: absorbance of the sample, A_{st}: absorbance of the standard, C_{st}: Concentration of standard

Statistical Analysis:

Statistical analyses were performed using IBM SPSS Statistics software, version 26.0. Mean and standard deviation (mean ± SD) were used to express data. The Shapiro–Wilk test was applied for testing the distribution of continuous variables. The independent samples t-test for normally distributed variables and the Mann–Whitney U test for those that were not normally distributed were applied for comparisons between groups. Correlations between oxidative stress markers and anthropometric or biochemical parameters were tested using Spearman's rank-order correlation. P-value less than 0.05 is considered statistically significant, less than 0.01 is highly significant, and greater than 0.05 is not significant.

3. RESULTS

Table 1 summarizes demographic characteristics of the studied groups. No significant difference between the groups was observed in age, height, and SBP ($p > 0.05$). However, body weight, BMI, WC, and DBP were significantly higher in obese groups compared to control group ($p < 0.05$).

Biochemical and oxidative stress parameters are presented in Table 2. Obese participants demonstrated significantly elevated levels of FBG, urea, uric acid, TC, TG, LDL-C, VLDL-C, liver enzymes (ALT, AST, ALP, and GGT), hs-CRP, FI, and HOMA-IR values compared to non-obese group ($p < 0.05$). In contrast, HDL-C concentrations were significantly lower among obese subjects ($p < 0.001$).

Regarding trace elements, Zn and Cu levels were elevated in obese males ($p = 0.002$ and $p < 0.001$, respectively), while Mg concentrations are significantly reduced ($p < 0.001$). Antioxidant enzyme activities, including GST, catalase, and SOD, were markedly lower in obese group ($p < 0.05$ and $p < 0.001$, respectively)

Table 1: Demographic Parameters between Obese and Non-Obese Adult Males

Variable(unite)	Control Group	Obese Group	p-value
Age (years)	28.6±6.9	30.6±7.4	0.201
Weight (kg)	66.5±7.4	101.8***±12.5	<0.001
Height (cm)	173±6.2	173±7.8	0.984
BMI (kg/m²)	22.2±1.7	34***±3	<0.001
WC (cm)	82.6±5.6	112.8***±7.4	<0.001
SBP (mmHg)	120.5±8.6	123.8±9.6	0.314
DBP (mmHg)	72.6 ±9.1	80***±11.01	0.001

p-value: <0.05 (*) is significant, <0.01 is high significant (**), p < 0.001 (***) statistical very high significant and >0.05 is not significant.

Table 2: Comparison of Biochemical and Oxidative Stress Parameters Between Obese and Non-Obese Adult Males

Variable(unite)	Control Group	obese Group	p-value
FBG (mg/dL)	82.5 ±6.1	90*** ±9.4	<0.001
Urea (mg/dL)	25±7	28.3**±6.3	0.007
Creatinine (mg/dL)	0.95±0.1	0.9±0.1	0.162
Uric acid (mg/dL)	5.1±0.96	6.2***±1	<0.001
TC (mg/dL)	137.4±24	204.5*±219.6	0.037
TG (mg/dL)	93±36.5	202.3***±114.8	<0.001
HDL-C (mg/dL)	41.8±7.9	38.4***±33.4	<0.001
LDL-C (mg/dL)	77.1±20.4	99.8***±30.4	<0.001
VLDL-C (mg/dL)	18.6±7.3	40.5***±23	<0.001
ALT (U/L)	17±5.5	35***±14.1	<0.001
AST (U/L)	21.3±4.6	27.2***±7.6	<0.001
ALP (U/L)	84.8±23.5	95.7*±27	0.047
GGT (U/L)	14.7±6.3	33.4***±18.8	<0.001
hs-CRP (mg/L)	0.8±0.9	3.8***±3.3	<0.001
FI (µIU/mL)	6.1±2.6	23.8***±14.8	<0.001
HOMA-IR	1.3±0.6	5.4***±4	<0.001
Zn(µg/dl)	57.01±32.8	85.7**±59.1	0.002
Cu(µg/dl)	140.5±96.7	238.8***±108.1	<0.001
Mg(mg/dL)	3±0.5	2**±0.7	<0.001
GST (µM)	105.5±41.4	84.6±51	0.005
Catalase (KU/L)	13.3 ±3.54	9.1*** ±2.1	<0.001
SOD	0.2±0.04	0.17***±0.05	<0.001

p-value: <0.05 (*) is significant, <0.01 is high significant (**), p < 0.001 (***) statistical very high significant and >0.05 is not significant.

As shown in Table 3, GST activity demonstrated significant negative correlations with several anthropometric and metabolic parameters. Specifically, it was inversely correlated with body weight, BMI, and WC. Among biochemical markers, GST was negatively associated with FBG, TG, VLDL-C, ALT, GGT, FI, and HOMA-IR. No significant correlations were observed between GST and HDL-C, LDL-C. However, no significant relationship was observed between GST and trace elements such as Zn, Cu, or Mg level. A positive correlation was noted between GST and catalase and SOD.

SOD activity also exhibited strong negative correlations with several markers of obesity and metabolic disturbance. It was inversely correlated with weight, BMI, and WC. SOD was negatively correlated with TG, TC, VLDL-C, ALT, AST, GGT, hs-CRP, FI, HOMA-IR, Zn and Cu concentration. A significant positive correlation was found with HDL-C and with both catalase and GST.

Catalase activity demonstrated the most pronounced inverse correlations with obesity markers: weight, BMI, and WC. It also negatively correlated with FBS, TC, TG, HDL-C, LDL-C, VLDL, ALT, AST, GGT, hs-CRP, FI, and HOMA-IR. Concerning trace elements, catalase showed a significant negative correlation with Cu level and a significant positive correlation with Mg concentration. No significant correlation was found with zinc. Catalase also had positive correlations with SOD and GST.

Table 3: Correlation Coefficients Between Oxidative Stress Markers and Clinical/Biochemical Parameters

Variable(unite)	GST (µM)		SOD		Catalase (KU/L)	
	r	p-value	r	p-value	r	p-value
Age (years)	0.004	0.967	-0.078	0.466	-0.141	0.184
Weight (kg)	-0.384***	<0.001	-0.396***	<0.001	-0.503***	<0.001
Height (cm)	-0.330***	0.001	-0.049	0.647	0.012	0.910
BMI (kg/m²)	-0.255*	0.015	-0.375***	<0.001	-0.514***	<0.001
WC (cm)	-0.284**	0.007	-0.407***	<0.001	-0.571***	<0.001
FBG (mg/dL)	-0.228*	0.031	-0.169	0.111	-0.335***	0.001
Urea (mg/dL)	0.075	0.485	0.047	0.661	0.062	0.565
Creatinine (mg/dL)	-0.067	0.529	0.099	0.355	0.148	0.164
Uric acid (mg/dL)	-0.157	0.140	-0.166	0.118	-0.127	0.233
TC (mg/dL)	-0.071	0.505	-0.282**	0.007	-0.507***	<0.001
TG (mg/dL)	-0.266*	0.011	-0.459***	<0.001	-0.551**	<0.001
HDL-C (mg/dL)	0.139	0.191	0.271**	0.010	0.301**	0.004
LDL-C (mg/dL)	-0.015	0.886	-0.185	0.081	-0.369***	<0.001
VLDL-C (mg/dL)	-0.266	0.011	-0.459***	<0.001	-0.551***	<0.001
ALT (U/L)	-0.222*	0.036	-0.399***	<0.001	-0.447***	<0.001
AST (U/L)	-0.087	0.414	-0.308**	0.003	-0.249*	0.018
ALP (U/L)	-0.085	0.426	-0.041	0.698	-0.098	0.357
GGT (U/L)	-0.236*	0.025	-0.359***	0.001	-0.418***	<0.001
hs-CRP (mg/L)	-0.103	0.333	-0.235*	0.026	-0.296**	0.005
FI (µIU/mL)	-0.254*	0.016	-0.409***	<0.001	-0.513***	<0.001
HOMA-IR	-0.260*	0.013	-0.396***	<0.001	-0.513***	<0.001
Zn(µg/dl)	-0.056	0.603	-0.235*	0.026	-0.187	0.078
Cu(µg/dl)	-0.171	0.107	-0.408***	<0.001	-0.392***	<0.001
Mg(mg/dL)	0.092	0.387	0.145	0.172	0.414***	<0.001
GST (µM)	1	-	0.372***	<0.001	0.238*	0.024
Catalase (KU/L)	0.238*	0.024	0.392***	<0.001	1	-
SOD	0.372***	<0.001	1	-	0.392***	<0.001

*Correlation at 0.05 is statistically significant, **Correlation at 0.01 is high significant, and ***Correlation at 0.001 is very high significant

4. DISCUSSION

This study aimed to evaluate the impact of obesity on oxidative–antioxidant balance and trace element status in adult males in Zakho City and as well as to investigate the association of these biomarkers with anthropometric and biochemical parameters. The findings revealed a substantial oxidative imbalance and altered trace element levels in obese individuals compared to non-obese controls.

Obese participants had significantly higher BMI, WC, DBP, FBG, lipid profile (TC, TG, LDL-C, and VLDL), liver enzymes (ALT, AST, ALP, and GGT), hs-CRP, FI, and HOMA-IR. These results were in agreement with previous research indicating that obesity was strongly associated with insulin resistance, dyslipidemia, hepatic stress, and systemic inflammation(Liu *et al.*, 2021; Rohm *et al.*, 2022). The elevated hs-CRP observed in this study support the presence of chronic inflammation, a well-recognized characteristic of obesity-associated metabolic dysfunction *(Lund et al.*, 2020).

A major focus of this study was the evaluation of oxidative stress and antioxidant defense. All three measured antioxidant enzymes GST, SOD, and catalase were significantly lower in obese individuals. This finding suggests a compromised antioxidant defense in response to increased ROS, likely resulting from excess adipose tissue and the consequent metabolic overload*(Yu et al*., 2023). The reduction in antioxidant activity reflected a failure of the body to adequately counter oxidative stress, which may contribute to the progression of obesity-related complications such as insulin resistance and

cardiovascular disease(Idan & Mohamoud, 2024; Świątkiewicz *et al.*, 2023).

Among the antioxidant enzymes, GST activity was significantly reduced in obesity and negatively correlated with weight, BMI, WC, FBS, TG, VLDL-C, ALT, GGT, FI, and HOMA-IR. These results were suggested lower GST activity was associated with worsening metabolic control and liver dysfunction. Previous studies have shown that GST played a role in detoxifying lipid peroxidation products and other oxidative metabolites, and its reduced activity may have enhanced cellular vulnerability to oxidative injury(Awasthi *et al.*, 2017).

SOD activity also decreased significantly in obese individuals and was negatively associated with multiple parameters, including weight, BMI, lipid profile, liver enzymes, and inflammatory and insulin-related markers. In particular, SOD showed a significant negative correlation with Zn and Cu levels, which may be explained by altered trace element homeostasis under inflammatory conditions. While Zn and Cu are cofactors for Cu/Zn-SOD, their elevated serum levels in obesity may reflect redistribution due to stress or altered absorption, rather than improved enzymatic function(Franco & Canzoniero, 2024). This paradoxical increase in trace element levels alongside decreased SOD activity was suggested that oxidative stress, rather than element deficiency, may have driven enzyme suppression.

Catalase, the third antioxidant enzyme assessed, showed the strongest inverse correlations with obesity indicators and biochemical markers. In addition to its negative correlation with weight, BMI, lipid profile, liver enzymes, and insulin resistance. On the other way catalase was positively correlated with magnesium levels. Magnesium is essential for numerous enzymatic processes, including those involving antioxidant defense, and hypomagnesemia has been frequently reported in obesity and type 2 diabetes (Piuri *et al.*, 2021). The positive relationship between catalase and magnesium suggested that adequate Mg levels may help maintain antioxidant capacity. Conversely, catalase was negatively associated with copper, reinforcing the hypothesis that excess copper contributed to oxidative stress(Sielska *et al.*, 2024).

Regarding trace elements, obese individuals had significantly elevated Zn and Cu levels, but significantly reduced Mg levels. Although Zn and Cu are essential cofactors in antioxidant enzyme systems, their elevated concentrations in this context may be part of an acute-phase response to inflammation rather than indicative of enhanced antioxidant defense(Li *et al.*, 2023; Olechnowicz *et al.*, 2018). The consistent finding of lower Mg level in the obese group aligns with existing literature and may be related to impaired glucose metabolism, reduced insulin sensitivity, and pro-inflammatory status (Piuri *et al.*, 2021; Xu *et al.*, 2024).

The positive correlations among GST, SOD, and catalase observed in the study was suggested a coordinated antioxidant response to manage oxidative stress. Their simultaneous decline in the obese group was highlighted a systemic impairment of antioxidant defense, which likely contributed to the pathophysiology of obesity and its associated metabolic complications.

CONCLUSION

This study demonstrated that obesity was associated with reduced antioxidant enzyme activities and altered trace element levels. Obese individuals showed lower levels of GST, SOD, and catalase, alongside increased metabolic and inflammatory markers. Significant correlations between antioxidant enzymes such as GST, SOD, and catalase and clinical parameters including BMI, lipid profile, and inflammatory markers suggested that oxidative stress plays a central role in the development of obesity-related complications. The imbalance in trace elements, especially decreased Mg and increased Cu and Zn, further highlights the oxidative burden in obesity. These findings was suggested that monitoring antioxidant status and trace elements might have valuable in managing obesity and its metabolic risks.

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Ethical Approval:

Ethical approval for this research is approved in November 2024 from the Research Ethics Committee, College of Medicine, University of Zakho (Ref: NOV2024/UOZE28).

Authors Contribution:

The project framework was devised by Assistant Professor L.Y.M., who also offered guidelines during the research period. S.A.A., managed all facets of sample collection, biochemical testing, data analysis, and literature research. The manuscript of this study was composed by S.A.A., Both the author and the supervisor evaluated and ratified the final version.

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