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EVALUATION OF SERUM HEPCIDIN LEVEL AND ITS RELATIONSHIP WITH CARDIOVASCULAR RISK FACTORS IN CHOLECYSTITIS PATIENTS IN ZAKHO CITY, IRAQ: *A CROSS-SECTIONAL STUDY*

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ABSTRACT

Background: Background: Hepcidin is an interleukin-6-induced liver derived acute phase protein which is part of the systemic iron metabolism and is becoming more frequently associated with being the mediator connecting chronic inflammation and atherosclerosis. Cholecystitis is a biliary disease which shares many risk factors for cardiovascular disease such as obesity, dyslipidemia and the metabolic syndrome; however the role of hepcidin in serum in this disease and its association with cardiovascular risk factors is not known.

Objectives: To determine the serum level of hepcidin in patients with cholecystitis, compare them with healthy subjects and correlate them with various cardiovascular risk factors.

Methods: Cross-sectional research was conducted on 100 patients who had been suffering from acute, chronic or acute on chronic cholecystitis and 50 apparently healthy individuals who were used as control. Demographic, anthropometric and clinical parameters were recorded along with estimation of serum hepcidin using ELISA method along with Liver function test, Renal function test, lipase/amylase, inflammatory markers (CRP), RBS, hematological and Iron Study (serum iron, UIBC, TIBC and transferrin saturation). Some patients were further divided based on bile bacterial culture (growth vs. no growth) and estimation of total oxidant status, total antioxidant status and protein carbonyl done compared to another group of healthy controls. Data analysis was performed using descriptive statistics and Mann-Whitney U & Kruskal-Wallis test for group comparison while Spearman and Pearson's correlation coefficients were used for correlation between serum hepcidin and continuous variables.

Results: Population: The study group was comprised of 83% females and 17% males, having a mean age of 44.1 ± 13.6 years with a mean body mass index (BMI) of 27.8 ± 4.3 kg/m², of whom 46% were found to be overweight and 26% were obese. There were 94% patients suffering from gallstones, 51% cases were chronic cholecystitis, 26% acute on chronic and 23% were acute cholecystitis. There was a significantly high value of serum hepcidin (1780.4 ± 524.9 ng/mL) in cholecystitis patients as compared to control subjects (122.6 ± 151.2 ng/mL; $p < 0.001$). Serum hepcidin was negatively associated with serum lipase (Spearman's $\rho = -0.23$, $p = 0.021$) but did not show any significant relationship with age, BMI, CRP, random blood sugar and indices of iron status (all $p > 0.05$). Significantly higher levels of hepcidin were found in hypertensive patients (1924.0 ± 450 ng/mL) as compared to normotensive patients (1746.7 ng/mL; $p = 0.046$), while there was no statistically significant difference with respect to gender, smoking, physical activity, BMI, disease phase, gallstone and bile culture status (all $p > 0.05$). Total oxidant status and total antioxidant status were significantly different among groups of bile-culture positive, negative and controls ($p < 0.001$ and $p = 0.036$.

Conclusion: Hepcidin serum levels were found to be substantially increased in cholecystitis due to its nature of being an inflammatory acute-phase protein in both hepatic and biliary systems. This specific correlation of serum hepcidin levels with only hypertension, and not the other factors related to cardiometabolic risk, indicates that this is mainly due to the inflammatory process and not the entire cardiometabolic risk profile, which needs to be confirmed in larger studies.

KEYWORDS*:* Hepcidin; Cholecystitis; Cardiovascular risk factors; Inflammation; Iron metabolism; C-reactive protein; Oxidative stress.

INTRODUCTION

Hepcidin is a 25-amino acid peptide hormone produced by hepatocytes and coded for by the HAMP gene. It is the master regulator of systemic iron balance, as it binds the cellular iron exporter ferroprotein expressed on enterocytes, macrophages and hepatocytes, initiating the internalization and degradation of the latter protein and thus suppressing the absorption of iron from the diet and the mobilization of recycled iron into the bloodstream

(Ganz and Nemeth, 2011). In addition to its physiological function of regulating iron metabolism, hepcidin functions as a type II acute phase protein, as its transcription in the liver is highly up-regulated by interleukin-6 (IL-6) in the presence of infection and inflammation, leading to hypoferremia that is a hallmark of the anemia of inflammation (Nemeth et al., 2003).

In recent years, increasing evidence emerged showing the role of hepcidin in the connection between chronic low-grade inflammation, iron homeostasis and cardiovascular diseases. Higher levels of hepcidin have been found to correlate with carotid intima-media thickness and plaque burden, poor prognosis after acute coronary syndrome, and cardiovascular mortality in hemodialysis patients (Galesloot et al., 2014; Li et al., 2017). Proposed mechanisms include the iron trapping action of hepcidin in macrophages in the plaque, resulting in increased oxidative stress, foam cell formation and plaque instability, and a broader role as an acute phase response product following the inflammatory cascade initiated by IL-6 which causes direct damage to the vascular endothelium (Rosenblum, 2023). Significantly, hepcidin seems to be more sensitive to predicting cardiovascular risk in the setting of inflammation, such as rheumatoid arthritis, chronic kidney disease and bacteremia, than in otherwise healthy subjects without signs of inflammation (Abdel-Khalek et al., 2011).

Cholecystitis, which refers to the inflammation of the gallbladder most often caused by the obstruction of the cystic duct by a gallstone, is itself an inflammatory condition both locally and systematically. The expression of hepcidin has been shown in the biliary epithelial cells of the gallbladder and bile ducts, and to be inducible by IL-6, with the level of hepcidin further upregulated in the mucosa of the gallbladder in patients with acute cholecystitis, suggesting the role of biliary hepcidin as a locally-specific antimicrobial protein along with its hepatic origin (Strnad et al., 2011). The disease of gallstones and cholecystitis also share common risk factors and epidemiological links to cardiovascular diseases: obesity, insulin resistance and the metabolic syndrome are recognized risk factors for cholesterol gallstones and atherosclerotic disease (Shaffer, 2005; Stinton and Shaffer, 2014; Sun et al., 2022); several prospective cohorts have also reported that the presence of gallstone disease predicts coronary heart disease independently of classical cardiovascular risk factors (Zheng et al., 2016; Olaiya et al., 2013).

Given the connections among gallbladder inflammation, iron metabolism and cardiometabolic risk factors, the present study was designed to (i) assess the levels of hepcidin in serum of patients with cholecystitis and healthy controls and (ii) systematically investigate the associations of hepcidin in serum with anthropometric, biochemical and clinical parameters, including age, body mass index (BMI), hypertension, diabetes, ischemic heart disease, smoking, physical activity, C-reactive protein (CRP), glucose levels and other metabolic parameters, iron metabolism, and hepatobiliary and pancreatic enzymes and oxidative stress parameters. To our best knowledge, this is the first study conducted in the Kurdistan Region of Iraq to comprehensively assess the serum hepcidin level in patients with cholecystitis and its association with inflammatory, cardiometabolic, hepatobiliary and iron status and oxidative stress parameters.

MATERIALS AND METHODS

Study Design and Population

A cross-sectional study was conducted between 1 March 2025 and 1 December 2025 in Zakho City, Kurdistan Region, Iraq. The study included 100 patients diagnosed with cholecystitis (acute, chronic, or acute-on-chronic) and 50 apparently healthy subjects as a comparison group. Patients were recruited from Zakho General Hospital and selected private hospitals in Zakho City. The diagnosis of cholecystitis was established based on clinical assessment by general surgeons, laboratory investigations, and abdominal ultrasonography performed by radiologists. Patients who underwent laparoscopic cholecystectomy were included in the study, and the surgical procedures were performed by general surgeons

Body Mass Index (BMI):

Body Mass Index (BMI) was calculated for all participants according to World Health Organization (WHO) guidelines. Participants were classified into four categories based on their BMI: underweight (BMI <18.5 kg/m²), normal weight (BMI 18.5–24.9 kg/m²), overweight (BMI 25.0–29.9 kg/m²), and obese (BMI ≥30.0 kg/m²

Inclusion and exclusion criteria

Criteria for participants were defined using those having been diagnosed with cholecystitis based on their clinical, laboratory, and ultrasonic results. Those who agreed to take part in the research and gave consent to do so were taken into account for participation. Patients suffering from other infections apart from cholecystitis, those taking immunosuppressive drugs, or those undergoing chemotherapy were excluded from the research.

Sample collection and processing:

Bile was aseptically collected from patients who were scheduled for cholecystectomy due to cholecystitis from a general surgeon, and approximately 10 mL of it was put in a sterile plain tube for microbial examination. These samples were immediately sent to the microbiology laboratory for culture of bacteria. Biles were cultured and patients were categorized either as positive or negative for bile cultures depending on the presence or absence of bacterial growth. Bile samples were inoculated on blood agar, nutrient agar, and MacConkey agar and then incubated. After incubation, the growth of the bacteria was identified depending on the colony morphology and Gram stain. Identification of bacterial isolates was done by conventional methods first and later using the VITEK system of automated identification (bioMérieux, France). Furthermore, approximately 8 mL of venous blood sample was collected from the study participants and out of it 5 mL was collected in a gel tube which was

centrifuged at 3,000 rpm for 10 minutes to get the serum while the other 3 mL was taken in EDTA tubes for CBC estimation.

Biochemical and hematological analyses

Serum was analyzed by COBAS 311 biochemical auto-analyzer (Roche, Diagnostics, Switzerland). The measurements for total, direct, and indirect bilirubin, alanine aminotransferase (GPT), aspartate aminotransferase (GOT), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), lipase, amylase, lactate dehydrogenase (LDH), C-Reactive Protein (CRP), random blood sugar (RBS), serum albumin, and iron status including serum iron, unsaturated iron binding capacity (UIBC), total iron binding capacity (TIBC) and transferrin saturation were done from serum. Peripheral blood specimens were collected in potassium-EDTA tubes, and a complete blood count (CBC), including white blood cell (WBC), hemoglobin (Hb), and platelet (PLT) counts, was performed using a Sysmex XP-300 automated hematology analyzer (Sysmex Corp., Japan) .

Enzyme-linked immunosorbent assay (ELISA)

Serum levels of total oxidant status (TOS), total antioxidant status (TAS), protein carbonyl, and hepcidin were quantified using ELISA kits (Bioassay Technology Laboratory, USA) as per manufacturer's guidelines. In a predefined subset, biomarkers were also tested among bile culture-positive patients, bile culture-negative patients, and healthy control individuals (n = 30 for each group) to evaluate the systemic oxidative stress caused by biliary infection. In brief, serum samples along with the standards were applied into the designated wells and the procedure continued as per the specific kit protocol. Following incubation, washing of wells was done, substrate solutions were added, and further incubated in darkness. Enzyme reaction was stopped by adding stop solution and absorbance was detected at 450 nm in a microplate reader. Concentration of TOS, TAS, protein carbonyl, and hepcidin was calculated from the standard curve and expressed in pg/mL.

Statistical Analysis

Continuous variables were expressed as mean ± standard deviation (SD) and range, while categorical variables were presented as frequencies and percentages. Associations between serum hepcidin and continuous variables were assessed using Spearman's rank correlation and Pearson's correlation coefficients. Differences between two independent groups were evaluated using the Mann–Whitney U test, while the Kruskal–Wallis test was used for comparisons among three or more groups. Serum hepcidin levels between patients and controls were compared using the Mann–Whitney U test and independent-samples t-test with Welch correction. A two-tailed $p < 0.05$ was considered statistically significant.

2.4 Ethical approval

Ethical approval for this study was obtained from the Medical and General Health Research Ethics Committee of the Ministry of Health, Duhok Directorate General of Health, under reference number (31072024-6-8). All participants provided informed consent before enrolment in the study, and the research was conducted in accordance with the ethical principles of the Declaration of Helsinki.

RESULTS

Demographic and Clinical Characteristics

There were one hundred cases of cholecystitis involved in this study (Table 1). Predominantly, there were more females (83%) and the average age was 44.1 ± 13.6 years (age range 14-74 years). The average BMI was 27.8 ± 4.3 kg/m2 and based on the WHO standard classification, 28% of the subjects had normal BMI, 46% were overweight and 26% were obese. Presence of gallstones was seen in 94% of the subjects. Of the total number of patients, 51% had chronic cholecystitis, 26% had acute-on-chronic and 23% had acute cholecystitis. There were 10% patients who were active smokers and 30% had a sedentary habit. As far as comorbid conditions associated with the cardiovascular system are concerned, 19% patients had a history of hypertension, 9% had diabetes mellitus, and 4% had ischemic heart disease.

Table 1. Demographic and clinical characteristics of the study cohort (n = 100)

Characteristic	Category	n (%) / Mean ± SD
Age (years)	Mean ± SD (range)	44.1 ± 13.6 (14–74)
Gender	Female	83 (83.0%)
	Male	17 (17.0%)
BMI (kg/m²)	Mean ± SD	27.8 ± 4.3
BMI category	Normal (18.5–24.9)	28 (28.0%)
	Overweight (25–29.9)	46 (46.0%)
	Obese (≥30)	26 (26.0%)
Disease stage	Chronic	51 (51.0%)
	Acute-on-chronic	26 (26.0%)

Characteristic	Category	n (%) / Mean ± SD
	Acute	23 (23.0%)
Gallstones present	Yes	94 (94.0%)
	No	6 (6.0%)
Smoking	Non-smoker	90 (90.0%)
	Smoker	10 (10.0%)
Physical activity	Active	70 (70.0%)
	Sedentary	30 (30.0%)
History of Hypertension	Yes	19 (19.0%)
History of Diabetes mellitus	Yes	9 (9.0%)
History of Ischemic heart disease	Yes	4 (4.0%)
Positive bile culture	Yes	30 (30.0%)

Biochemical Profile

Descriptive statistics for the full biochemical panel are shown in Table 2. Serum hepcidin ranged widely (37.5–2512 ng/mL; mean 1780.4 ± 524.9 ng/mL), as did several acute-phase and hepatobiliary markers, notably CRP (mean 18.4 mg/dL, maximum 323 mg/dL) and GGT (mean 51.4 IU/L, maximum 1737 IU/L), reflecting the heterogeneous inflammatory severity across acute and chronic presentations in this cohort.

Table 2. Descriptive statistics of anthropometric, biochemical and haematological parameters (n = 100).

Parameter	Mean ± SD	Median	Range
Age (years)	44.1 ± 13.6	44.0	14.0 – 74.0
BMI (kg/m²)	27.8 ± 4.3	27.6	19.5 – 44.5
Total bilirubin (mg/dL)	0.62 ± 0.70	0.40	0.20 – 4.70
Direct bilirubin (mg/dL)	0.26 ± 0.53	0.20	0.10 – 3.60
Indirect bilirubin (mg/dL)	0.33 ± 0.23	0.30	0.10 – 1.20
GPT / ALT (IU/L)	39.2 ± 91.1	19.0	5.0 – 632.0
GOT / AST (IU/L)	33.9 ± 61.5	21.5	10.0 – 522.0
ALP (IU/L)	90.8 ± 53.8	79.5	36.0 – 404.0
GGT (IU/L)	51.4 ± 177.3	20.5	3.0 – 1737.0
Lipase (IU/L)	32.7 ± 17.0	29.0	9.0 – 98.0
Amylase (IU/L)	63.7 ± 31.0	56.0	20.0 – 229.0
LDH (IU/L)	249.0 ± 114.8	221.5	86.0 – 989.0
CRP (mg/dL)	18.4 ± 52.9	2.6	0.2 – 323.0
RBS (mg/dL)	112.7 ± 40.7	100.5	71.0 – 384.0
Albumin (g/L)	4.26 ± 0.45	4.30	2.50 – 5.30
WBC (×10⁹/L)	8.84 ± 3.10	8.20	4.00 – 20.60
Haemoglobin (g/dL)	12.50 ± 1.56	12.60	9.00 – 15.90
Platelets (×10⁹/L)	262.4 ± 69.5	261.5	90.0 – 457.0
Serum hepcidin (ng/mL)	1780.4 ± 524.9	1854.5	37.5 – 2512.0
Serum iron (µg/dL)	44.3 ± 29.0	40.5	8.0 – 176.0
UIBC (µg/dL)	231.4 ± 79.4	226.5	34.0 – 428.0
TIBC (µg/dL)	275.7 ± 79.2	270.5	87.0 – 478.0
Transferrin saturation (%)	17.3 ± 12.8	15.6	2.7 – 77.4

Serum Hepcidin: Patients versus Controls

Serum hepcidin concentrations were markedly higher in patients with cholecystitis than in healthy controls (1780.4 ± 524.9 vs. 122.6 ± 151.2 ng/mL, respectively), representing an approximately **14.5-fold increase**. This difference was highly statistically significant according to both the Mann–Whitney U test and Welch's independent-samples *t*-test (both $p < 0.001$; Figures 1 and 2).

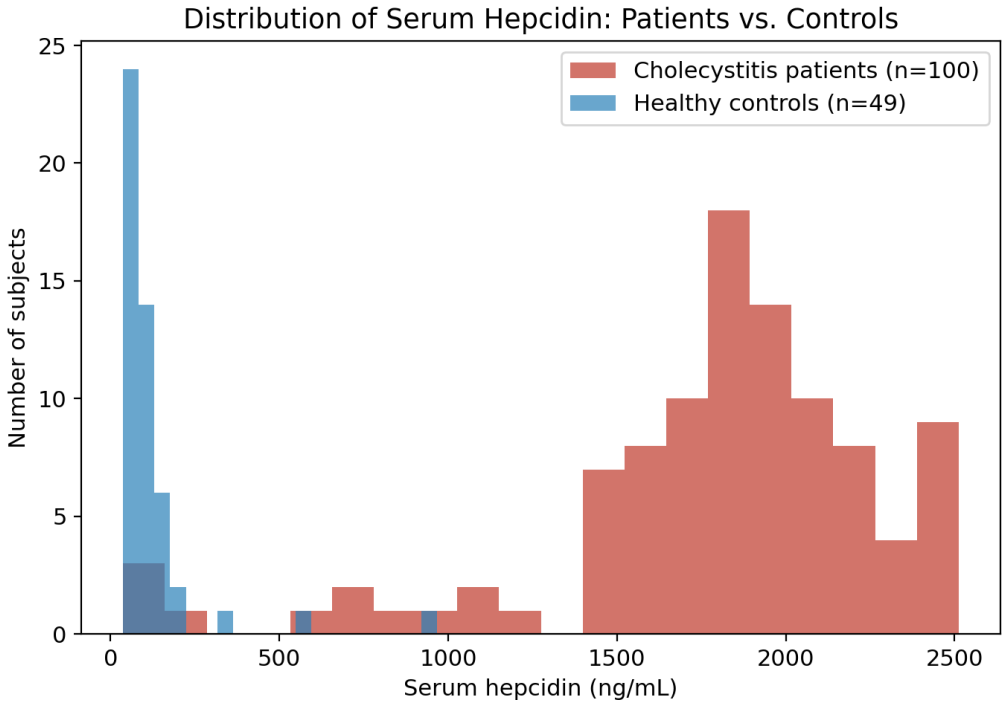

Figure 1. *Distribution of serum hepcidin concentrations in cholecystitis patients (n = 100) and healthy controls (n = 50).*

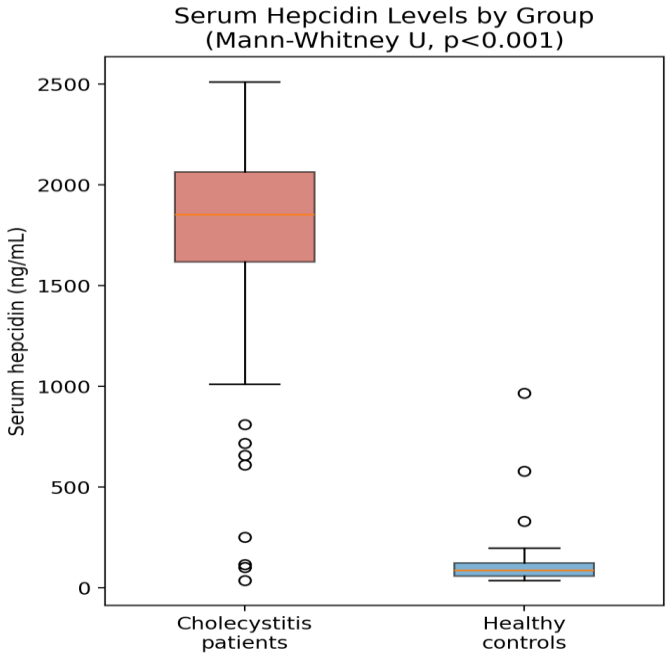

Figure 2. *Serum hepcidin levels in cholecystitis patients versus healthy controls (Mann–Whitney U test, $p < 0.001$). Box: interquartile range; line: median; whiskers: 1.5× IQR.*

Correlation of Serum Hepcidin with Continuous Variables

Table 3 summarises the correlation of serum hepcidin with all continuous anthropometric and biochemical variables. Serum hepcidin correlated significantly and negatively with serum lipase (Spearman's $\rho = -0.23$, $p = 0.021$; Pearson's $r = -0.24$, $p = 0.019$). A weak positive correlation with LDH reached significance by the Pearson but not the Spearman method ($r = 0.20$, $p = 0.043$; $\rho = 0.12$, $p = 0.257$), indicating limited robustness, likely driven

by a small number of markedly elevated LDH values. No significant correlations were observed between hepcidin and age, BMI, CRP, RBS, serum iron, UIBC, TIBC, transferrin saturation, haemoglobin, albumin, platelet count, or the remaining hepatobiliary enzymes (all $p > 0.05$). The overall correlation structure among all measured biochemical parameters is displayed as a heatmap in Figure 3, and hepcidin's relationship with lipase, CRP, age and BMI specifically is illustrated in Figure 4.

Table 3. Correlation of serum hepcidin with continuous anthropometric and biochemical parameters, ranked by statistical significance (Spearman's ρ).

Variable	Spearman's ρ	p (Spearman)	Pearson's r	p (Pearson)
Lipase	−0.233	0.021*	−0.236	0.019*
GGT	0.158	0.119	0.037	0.718
CRP	0.150	0.142	−0.004	0.969
LDH	0.116	0.257	0.205	0.043*
ALP	0.075	0.462	0.059	0.566
RBS	−0.075	0.464	−0.174	0.086
GOT / AST	0.060	0.556	0.107	0.295
Serum iron	0.059	0.558	0.090	0.373
Indirect bilirubin	−0.049	0.630	−0.059	0.564
Albumin	−0.049	0.641	−0.010	0.921
Platelets	0.045	0.659	−0.011	0.916
Haemoglobin	−0.038	0.708	−0.072	0.481
Transferrin saturation	0.034	0.740	0.079	0.433
GPT / ALT	0.029	0.774	0.095	0.350
Direct bilirubin	−0.029	0.781	0.083	0.420
Amylase	−0.026	0.799	−0.073	0.475
BMI	0.017	0.866	−0.013	0.898
Age	−0.014	0.888	−0.007	0.947
WBC	0.014	0.894	−0.063	0.546
TIBC	0.010	0.923	−0.064	0.524
UIBC	−0.006	0.954	−0.097	0.336
Total bilirubin	0.004	0.971	0.065	0.530

$P < 0.05$ was considered statistically significant.

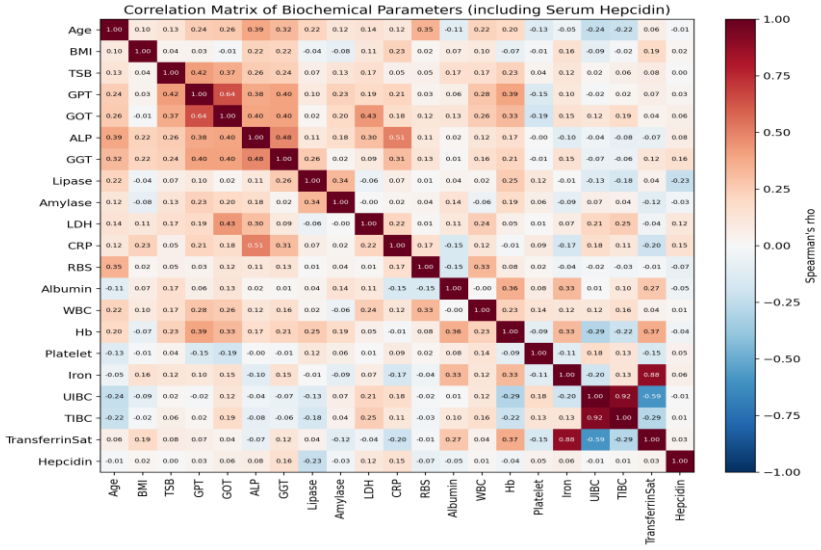

Figure 3. *Spearman correlation matrix of anthropometric and biochemical parameters, including serum hepcidin. Cell values are Spearman's ρ; colour intensity reflects correlation strength and direction*

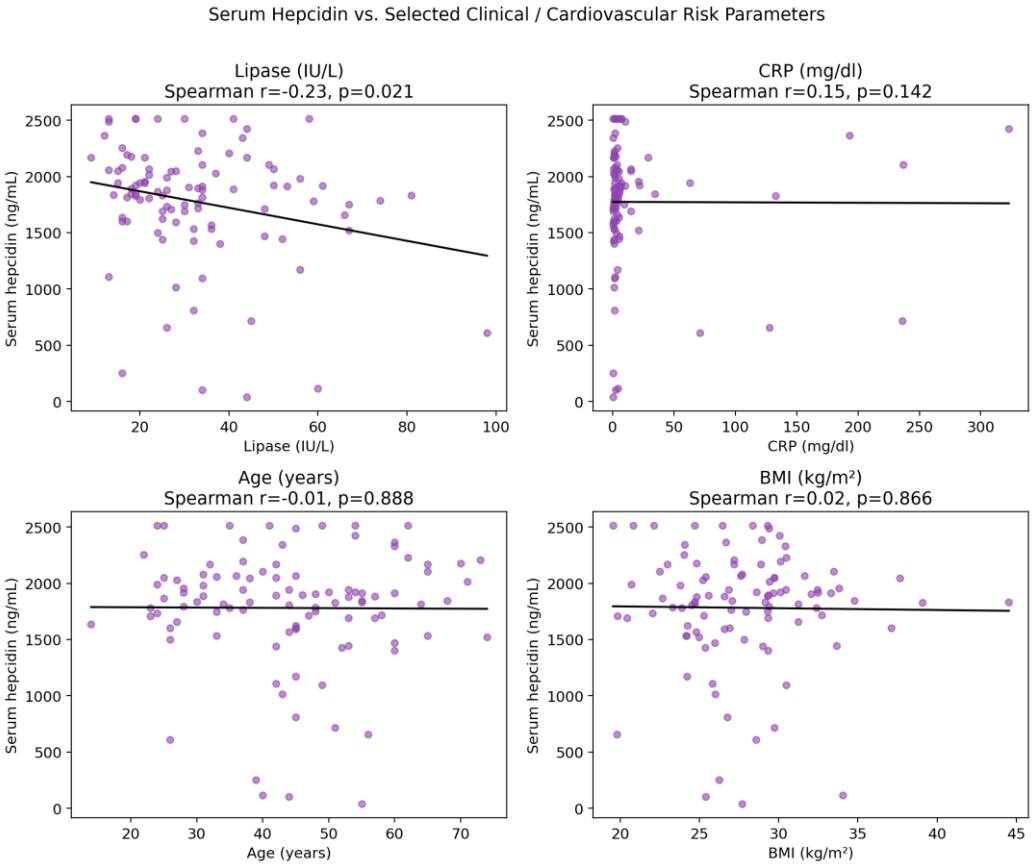

Figure 4. *Scatter plots of serum hepcidin against lipase, CRP, age and BMI, with linear trend lines and Spearman correlation statistics.*

Serum Hepcidin and Cardiovascular Risk Factors

When patients were stratified by categorical cardiovascular and clinical risk factors (Table 4), serum hepcidin was significantly higher in patients with a history of hypertension (mean 1924.0 ng/mL) than in normotensive patients (mean 1746.7 ng/mL; $p = 0.046$; Figure 7). No statistically significant differences in hepcidin were found by gender, smoking status, physical activity level, BMI category, diabetes, ischaemic heart disease, disease stage, gallstone presence, or bile culture status (all $p > 0.05$), although hepcidin trended higher in patients with a sedentary lifestyle and in those with bacterial growth on bile culture. Given the small numbers of patients with diabetes ($n = 9$) and ischaemic heart disease ($n = 4$), these comparisons were likely underpowered and should be interpreted with caution.

Table 4. Serum hepcidin levels according to categorical clinical and cardiovascular risk factors.

Variable	Group	n	Mean ± SD (ng/mL)	p-value
Gender	Female	83	1810.4 ± 489.7	0.253
	Male	17	1634.1 ± 669.5	
Smoking	Non-smoker	90	1790.3 ± 522.9	0.505
	Smoker	10	1691.5 ± 562.6	
Physical activity	Active	70	1790.6 ± 590.2	0.177
	Sedentary	30	1756.7 ± 332.9	
BMI category	Normal	28	1878.0 ± 423.3	0.698
	Overweight	46	1700.0 ± 613.4	
	Obese	26	1817.6 ± 442.8	
Hypertension	Yes	19	1924.0 ± n/r*	0.046
	No	81	1746.7 ± n/r*	

Variable	Group	n	Mean ± SD (ng/mL)	p-value
Diabetes mellitus	Yes	9	1572.9 ± n/r*	0.547
	No	91	1800.9 ± n/r*	
Ischaemic heart disease	Yes	4	1476.1 ± n/r*	0.666
	No	96	1793.1 ± n/r*	
Gallstone presence	Yes	94	1769.8 ± 530.7	0.637
	No	6	1946.7 ± 425.1	
Disease stage	Acute	23	1742.9 ± 578.1	0.901
	Chronic	51	1827.4 ± 414.9	
	Acute-on-chronic	26	1721.4 ± 665.7	
Bile culture	No growth	70	1756.9 ± 549.1	0.434
	Growth	30	1835.3 ± 467.6	

**SD not separately reported for these subgroup comparisons owing to small subgroup size (n = 4–9); mean values only are shown.*
± n/r: the standard deviation was not reported/not available.
*P values were determined using the Mann–Whitney U test.

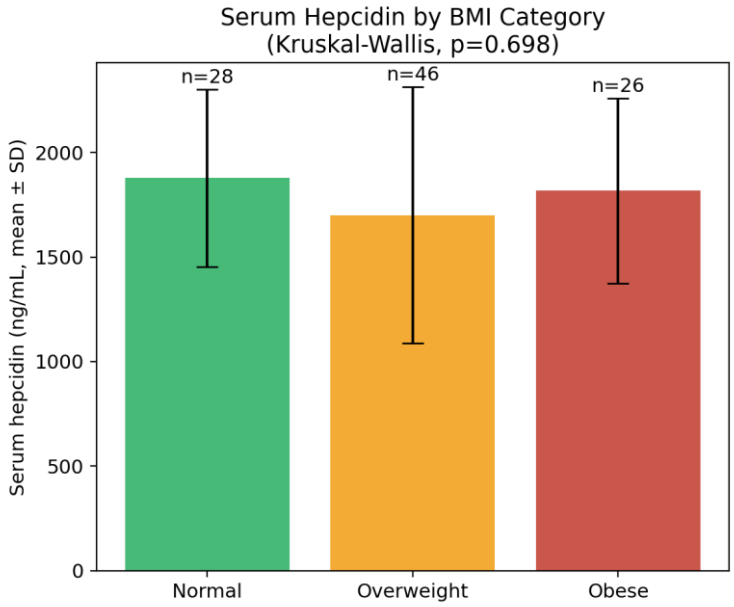

Figure 5. *Serum hepcidin by BMI category (Kruskal–Wallis test, p = 0.698). Bars show mean ± SD.*

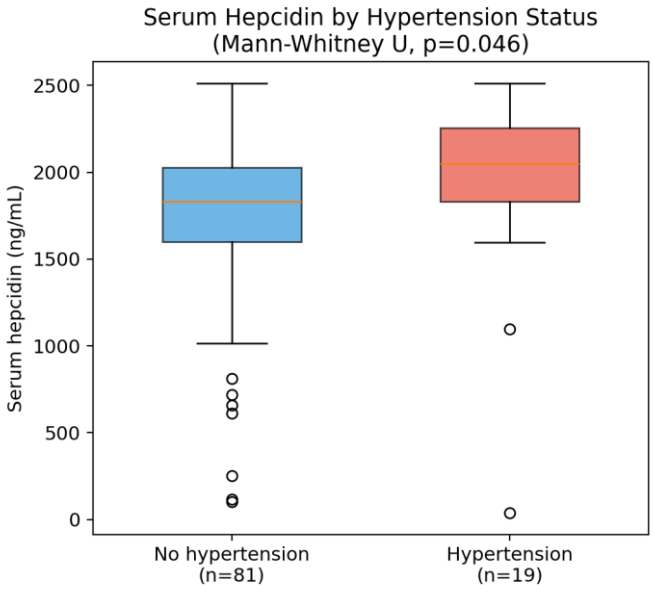

Figure 7. *Serum hepcidin by hypertension status (Mann–Whitney U test, p = 0.046).*

Oxidative Stress Markers by Bile Culture Status

Among the subgroup analysed for oxidative stress markers (30 participants per group), total oxidant status (TOS) differed significantly among bile-culture-positive, bile-culture-negative, and healthy-control groups (Kruskal–Wallis test, $p < 0.001$), with the lowest levels observed in culture-positive patients and the highest in healthy controls. Total antioxidant status (TAS) also differed significantly among the three groups ($p = 0.036$), showing a similar pattern, whereas protein carbonyl levels did not differ significantly ($p = 0.459$) (Table 5, Figure 6). These findings indicate differences in systemic oxidative and antioxidant status according to bile culture status; however, given the cross-sectional design, the observed pattern should not be interpreted as evidence of a causal relationship.

Table 5. Oxidant/antioxidant markers by bile bacterial culture status and healthy controls (n = 30 per group).

Parameter	Growth	No growth	Control	Kruskal–Wallis p
Total oxidant status (U/mL)	4.22 ± 1.34	5.48 ± 1.35	7.30 ± 4.28	< 0.001*
Total antioxidant status (U/mL)	9.84 ± 4.58	10.06 ± 4.68	16.25 ± 12.10	0.036*
Protein carbonyl (U/mL)	74.48 ± 25.09	69.03 ± 23.91	94.47 ± 73.82	0.459

**P < 0.05 was considered statistically significant.*

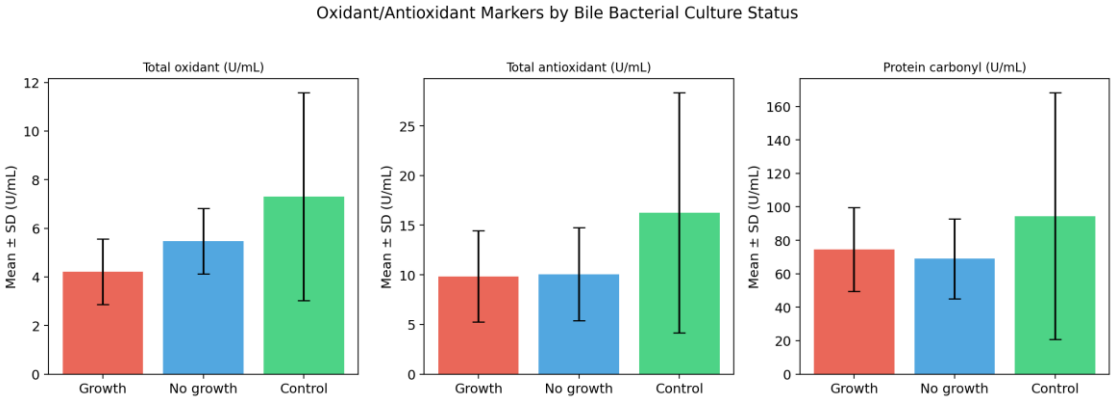

Figure 6. *Total oxidant status, total antioxidant status and protein carbonyl by bile bacterial culture status (growth vs. no growth) compared with healthy controls. Bars show mean ± SD.*

DISCUSSION

This study demonstrates a marked elevation of serum hepcidin in patients with cholecystitis compared with healthy controls, with patient levels approximately fourteen-fold higher than control values. This finding is

consistent with the well-established behavior of hepcidin as an IL-6-inducible type II acute-phase reactant protein (Nemeth et al., 2003; Nemeth and Ganz, 2023), and aligns specifically with evidence that biliary epithelial hepcidin expression is up-regulated in the gallbladder mucosa during acute cholecystitis, in addition to the systemic hepatic acute-phase response (Strnad et al., 2011). The magnitude of elevation observed here is considerably larger than that typically reported in chronic low-grade inflammatory states such as obesity or the metabolic syndrome, and more closely resembles the marked hepcidin induction described in acute bacteremia, where levels rise sharply with infection and fall again after successful antimicrobial treatment. More recent clinical evidence further supports the close relationship between hepcidin and acute infection. In patients with bacteremia, serum hepcidin was significantly elevated during infection and decreased following appropriate antimicrobial treatment, in parallel with changes in C-reactive protein, suggesting that hepcidin may function as a sensitive and treatment-responsive acute-phase marker (Koukoulas et al., 2022). Taken together, these findings support the interpretation that the marked hepcidin elevation observed in the present study may reflect an acute inflammatory response associated with cholecystitis, particularly in the presence of biliary infection. However, the magnitude of elevation should be interpreted cautiously because hepcidin concentrations can vary substantially according to disease severity, inflammatory status, iron status, and assay methodology.

With respect to cardiovascular risk, the principal positive finding was a significant association between elevated hepcidin and a history of hypertension. This observation is broadly consistent with a growing body of evidence linking hepcidin to vascular pathology, including its association with carotid plaque burden in postmenopausal women (Galesloot et al., 2014), its role as an independent predictor of mortality in patients with coronary artery disease (Li et al., 2017), and mechanistic work showing that hepcidin-mediated iron retention within macrophages can destabilase atherosclerotic plaque through enhanced oxidative stress and inflammatory cytokine release (Rosenblum, 2023). Chronic inflammatory conditions such as rheumatoid arthritis have similarly shown a direct link between hepcidin-driven anemia of inflammation and coronary atherosclerosis, independent of traditional lipid-based risk factors (Abdel-Khalek et al., 2011). It is plausible that, in the present cohort, hepatobiliary inflammation and possibly bile bacterial colonization act as a chronic inflammatory stimulus that, in hypertensive patients with pre-existing vascular dysfunction, produces a more pronounced hepcidin response – although the cross-sectional design cannot establish causality or direction of this association.

In contrast, serum hepcidin showed no significant relationship with age, BMI, glycaemia, or classical iron-status indices (serum iron, TIBC, UIBC, transferrin saturation) in this cohort. The absence of a hepcidin–BMI relationship is somewhat unexpected, since adipose tissue is itself a recognised source of hepcidin and obesity-related inflammation has previously been linked to elevated hepcidin in other populations; however, in the setting of acute biliary inflammation, the intensity of the local and hepatic acute-phase response to cholecystitis may simply overwhelm any more modest BMI-related signal, consistent with observations that hepcidin–cardiovascular associations are most apparent in settings of pronounced systemic inflammation rather than in metabolically driven, low-grade inflammatory states (Pechlaner et al., cited in Kali et al., 2017). Likewise, the lack of correlation between hepcidin and CRP in this cohort, despite both being acute-phase reactants, may reflect differences in the kinetics of their induction and clearance, or a ceiling effect given that hepcidin was already markedly and near-uniformly elevated across the great majority of patients.

The significant, if modest, inverse correlation between hepcidin and serum lipase is an interesting incidental finding. Because lipase elevation in this cohort likely reflects a spectrum from mild bystander pancreatic enzyme leakage to overt biliary pancreatitis, and hepcidin elevation reflects the intensity of the hepatobiliary acute-phase response, this inverse relationship may be a chance finding related to the specific case-mix of this cohort rather than a direct physiological interaction; it should be interpreted cautiously and would benefit from replication.

The gallstone disease literature provides broader epidemiological context for these findings. Several large prospective cohorts have shown that a history of gallstone disease independently predicts incident coronary heart disease, with hazard ratios in the range of 1.1–1.3 even after adjustment for obesity, diabetes and hypertension (Zheng et al., 2016; Olaiya et al., 2013), and gallstone disease itself shares upstream risk factors with cardiovascular disease, including obesity, insulin resistance and dyslipidaemia (Shaffer, 2005; Stinton and Shaffer, 2014; Sun et al., 2022). The present findings extend this literature by suggesting that hepcidin – as a biomarker sitting at the intersection of inflammation, iron handling and vascular biology – is one plausible mechanistic thread connecting biliary and cardiovascular disease, worthy of further targeted investigation, particularly given its selective association with hypertension observed here.

The oxidative stress findings in the bile-culture subgroup are more difficult to interpret, since both total oxidant and total antioxidant status were, unexpectedly, lower in culture-positive patients than in culture-negative patients and controls. This pattern could reflect consumption of oxidant/antioxidant substrates in the setting of intense local infective and inflammatory activity, assay or preservation artefacts related to sample handling in actively infected bile, or a genuinely blunted systemic oxidative response in the presence of overwhelming local antimicrobial and inflammatory activity within the biliary tree. Because this analysis was performed in a smaller subgroup using a separate control population, these results should be regarded as hypothesis-generating rather than definitive. . Therefore, the lower TOS and TAS observed in culture-positive patients may reflect altered systemic redox homeostasis during biliary infection, differences in inflammatory severity or timing of sampling, or methodological factors, rather than indicating a specific biological mechanism. Given the relatively small subgroup size and the use of a separate control population, these findings should be considered hypothesis-

generating rather than definitive and require confirmation in larger prospective studies using standardized sampling procedures and multiple complementary oxidative-stress biomarkers.

STRENGTHS AND LIMITATIONS

This study benefits from a reasonably large, well-characterised cohort of 100 cholecystitis patients with a comprehensive biochemical panel and a discrete healthy-control comparison group for hepcidin. However, several limitations warrant emphasis. First, the cross-sectional design precludes causal inference and does not allow assessment of temporal change in hepcidin relative to disease onset or resolution. *Second,* this dataset did not include a formal lipid profile (total cholesterol, LDL-C, HDL-C, triglycerides) or blood pressure measurements, both cornerstones of conventional cardiovascular risk assessment; cardiovascular risk factors here were therefore represented by a documented history of hypertension, diabetes and ischaemic heart disease together with BMI, smoking, physical activity and CRP, and future work should incorporate a full lipid and blood-pressure profile. Third, the subgroups with diabetes (n = 9) and ischaemic heart disease (n = 4) were small, limiting statistical power to detect true associations with hepcidin in these strata. Fourth, the oxidative stress analysis was performed in a separate subgroup with its own control population rather than being directly linked to the main cohort's hepcidin measurements, precluding a combined analysis of hepcidin and oxidative stress within the same patients.

CONCLUSION

Serum hepcidin is markedly elevated in patients with cholecystitis relative to healthy controls, consistent with its role as a hepatic and biliary acute-phase reactant. Among the cardiovascular risk factors examined, only a history of hypertension was significantly associated with higher hepcidin levels, while age, BMI, smoking, physical activity, diabetes, ischaemic heart disease and iron-status indices showed no significant relationship. These findings support the concept that hepcidin elevation in cholecystitis is primarily driven by the intensity of local and systemic inflammation rather than by the broader cardiometabolic risk profile, while raising the possibility of a specific hepcidin–hypertension link that merits further investigation in larger, prospective, multi-centre studies incorporating full lipid profiling, blood pressure measurement, and serial hepcidin sampling across the course of biliary inflammation and its resolution.

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Conflict of Interest

The authors declare that they have no conflicts of interest related to this study.

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