

Biochemical and histological profiles of gallstones in pre- and postmenopausal women

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Abstract

Objective: Cholesterol gallstones are frequently observed in postmenopausal women due to hormonal and metabolic alterations. Objective: To study differences in biochemical and histological gallbladder profiles between pre- and postmenopausal women with cholesterol gallstones.

Methods: A total of 100 patients with gallstones and 80 healthy controls. Inflammatory markers (CRP, IL-6, MDA), liver enzymes, lipids, and bilirubin were measured. Stone composition and gallbladder histology were examined.

Results: CRP, IL-6 and MDA were significantly increased in patients with gallstones compared to controls. IL-6 levels were increased in postmenopausal women, whereas MDA was higher in premenopausal patients. Serum total cholesterol and triglyceride levels were higher in postmenopausal patients. AST and ALT were raised in both groups. Magnesium levels were increased in postmenopausal gallstone patients, whereas no significant differences were found in the calcium, sodium, and potassium contents of the stones. Histology revealed severe inflammatory infiltration, RAS, mucosal hyperplasia, haemorrhage and muscular hypertrophy in chronic cholecystitis. Subepithelial foamy lipocytes, ulceration of the mucosa, vascular congestion, haemorrhage, thrombosis, and necrosis characterise acute cholecystitis. hypertrophic muscle bundles were commonly contiguous with these structures. The wall of gallbladders with cholesterol stones demonstrated a focal destruction of muscle fibres, congestion, widespread necrosis and an invasion by neutrophils and vacuolated cells.

Conclusion: Menopausal status strongly affects the biochemical and histopathological profiles of cholecystitis disease, with postmenopausal patients demonstrating greater metabolic and inflammatory disturbances, highlighting the importance of age- and hormone-specific approaches in diagnosis and management.

Keywords: Gallbladder, malondialdehyde, hyperplasia, chronic inflammation, Postmenopausal

Plain English Summary

Gallstones are solid concretions that form in the gallbladder and can cause pain, infection, and damage to gallbladder tissues. They are more common in women after the menopause stage due to loss of estrogen and metabolic changes. In the present study, we compared 100 women with cholesterol gallstones (pre- and postmenopausal) to 80 healthy women without gallstones. We recorded blood markers of inflammation, oxidative damage, liver function, and cholesterol, analysed the stones' chemical composition and the gallbladders' microscopic structure. Our results showed that women with gallstones had higher values of inflammation and oxidative stress markers in their blood compared to healthy women. Postmenopausal women had a higher degree of inflammatory activity, and premenopausal women had a greater level of oxidative stress. The levels of cholesterol and triglyceride were higher in the postmenopausal women, and this was reflected in the composition of the stones. The gallbladder tissue of patients was found to have heavy inflammation, damage to and thickening of tissue, damage to muscle fibres, and, in some cases, death of cells. These findings indicate that

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menopause has a significant influence on the development of gallstones by its effect on both blood composition changes and alterations of the gallbladder tissue

Background

Gallstone disease (cholelithiasis) is amongst the most frequently encountered gastrointestinal ailments worldwide and results from the formation of cholesterol-rich calculi within the gallbladder lumen (1). The aetiology of GB disease is multifactorial and comprises cholesterol supersaturation of bile, reduced gallbladder motility, and hypersecretion of mucin, all of which contribute to the nucleation of cholesterol crystals and gallstone formation (2, 3, 4).

It has also been largely reported that women, particularly during reproductive and postmenopausal periods, are at higher risk of developing gallstones, according to epidemiological studies (5). Most of this gender difference can be explained by the hormonal effect of oestrogen, which stimulates hepatic cholesterol secretion and inhibits bile acid synthesis, thus producing an increased taurocholate-like saturation of bile (6, 7).

Epidemiological studies demonstrate a higher prevalence of gallstones among women, particularly during pre-menopause and postmenopausal periods (5). This gender difference has largely been referred to hormonal effects, especially oestrogen, which changes hepatic cholesterol metabolism and bile composition (6, 7).

In Iraq, local reports have shown an increasing prevalence of gallstone disease. The study in Basrah found that the prevalence of adult patients with asymptomatic gallstones was 13.6% although the prevalence of the same was higher in women, the elderly, obese, and those with hypercholesterolemia (5, 8).

Despite this, very few studies have been conducted looking at both the biochemical and histological changes in cholelithiasis within different hormonal statuses together. Knowledge of the effects of systemic inflammatory and oxidative markers, liver function parameters, gallstone composition, and tissue-level alterations combined with the disease may provide new approaches towards the pathophysiology of the disease and the prevention strategies in high-risk populations (9).

Therefore, the objective of this study is to evaluate the inflammatory, oxidative, and hepatic biochemical parameters, chemical composition and histopathological characteristics of the gallbladder tissue of women with cholelithiasis. This comparative study between pre- and postmenopausal women is hoped to give us more insight into the effect of menopausal transition on gallstone aetiology and contribute to

the regional scientific knowledge and further expansion globally of this widely prevalent hepatobiliary entity.

Materials and Methodology

Study Design and Population

This comparative cross-sectional study was performed on 180 adult female patients with gallstones (18–74 years of age) attending a regional hospital in southern Iraq (Al-Sadr Hospital in Maysan Governorate). Participants were divided into the following subgroups according to their gallstone diagnosis and menopausal status: premenopausal women with gallstones (n=50), postmenopausal women with gallstones (n=50), premenopausal healthy women (n=40) and postmenopausal healthy controls (n=40). Gallstone disease was screened by abdominal ultrasonography, whereas menopausal status was considered as no menstruation for at least 12 months in a row without organic reasons and hormonal treatment. Each participant filled in a structured questionnaire to assess their medical history. Exclusion criteria were diabetes mellitus, thyroid disease, malignancies, autoimmune diseases, chronic liver disease, acute cholecystitis, hormone replacement therapy, or use of lipid-lowering drugs.

Sample size justification

The sample size (100 patients and 80 controls) was determined based on practical constraints, including the availability of eligible participants during the study period and the feasibility of biochemical and histological analyses. No formal a priori power analysis was conducted; however, the chosen sample size was comparable to or larger than those reported in similar regional studies and was considered sufficient for meaningful statistical comparisons.

Sample Collection

Blood Sample Collection

Blood sample collection was carried out after overnight fasting. The serum was centrifuged and kept at -80°C until biochemical analysis.

Gallstone Collection

Gallstones were collected from surgically resected gallbladders during cholecystectomy. After washing with deionised water, the stones were air-dried at room temperature for 4 weeks before analysis.

Gallbladder Tissue Sampling

Gallbladder tissue specimens were fixed in 10% neutral-buffered formalin, directly following surgical resection. Following standard processing, paraffin-embedded tissue blocks were cut at 5 µm and stained with haematoxylin and eosin to view histopathological changes (10).

Biochemical and Inflammatory Marker Assessment

Serum biochemical indexes were assayed by commercial kits from Roche (Germany) and tested on the COBAS c111 automatic analyser. The following parameters were measured: Lipid profile: Total cholesterol (T-Ch) and triglycerides (TG), Liver function enzymes: Alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), Bilirubin levels: Total, direct (DSB) and indirect (NSB). Inflammatory and oxidative stress markers, including C-reactive protein (CRP), interleukin-6 (IL-6) and malonaldehyde (MDA), were measured with enzyme-linked immunosorbent assay (ELISA) kits by following the manufacturer’s instructions.

Gallstone Typing and Chemical Composition Analysis

Gallstones were classified macroscopically based on colour and texture into three categories: Cholesterol stones (pale yellow/whitish), Pigment stones (black or dark brown), Mixed stones (brownish-yellow or green with visible laminations) (11).

Chemical composition was confirmed through stone digestion and analysis using colourimetric stone analysis kits. Levels of cholesterol, triglycerides, calcium, magnesium, sodium, and potassium were quantitatively assessed (12).

Histopathological Examination

The sections of the gallbladder stained with H&E were observed under a light microscope at 10× and 40× magnification. Pathological features such as mucosal papillary hyperplasia, lymphoplasmacytic infiltration, Rokitansky–Aschoff sinuses, muscle layer hypertrophy, haemorrhage, necrosis, and fibrinoid deposits were noted and compared between groups.

Statistical analysis

All statistical analyses were performed using SPSS version 27. Descriptive data were expressed as mean ± standard deviation (SD). Group differences were evaluated using general linear models and independent sample t-tests. A p-value of ≤ 0.05 was considered statistically significant (13).

Results

Histological and Biochemical Alterations in Gallbladders with Cholesterol Gallstones

Biochemical Parameters

Comparison of total cholesterol levels: Statistically, a highly significant difference was noted between both the hormonal groups of GS and the healthy control group in the levels of T-Ch. Postmenopausal patients exhibited significantly higher levels of T-Ch compared with controls (202.75±26.82 mg/dL and 158.98±12.28 mg/dL, respectively) (P≤0.001). Table 1

The level of TG was significantly higher in both pre- and post-menopausal groups when compared with healthy women. However, no difference was observed between the patient group, which suggests that high TG might be the cause for gallstone formation regardless of the menopausal group. Table 1

AST level was significantly higher in premenopausal patients compared with healthy women (P=0.001); however, no difference was found between postmenopausal patients and controls. This could be suggestive of a more conspicuous hepatic stress in younger gallstone patients. Table 1

Hepatic ALT levels were higher in the two groups of patients than in controls, with the more elevated serum ALT in premenopausal women. It implicates more severe liver cell injury in the premenopausal group. Table 1

Serum alkaline phosphatase (ALP) was increased in two groups of patients. But no statistical significance was found with the postmenopausal group when compared against the control (P=0.046). Table 1

Direct serum bilirubin (DSB) was significantly higher in premenopausal patients, while total serum bilirubin (TSB) and indirect bilirubin (NSB) were not statistically significantly different in patients with and without GS. Table 1.

Table 1: Comparison of biochemical parameters between patients and healthy women in premenopausal and postmenopausal age stages

Parameters	premenopausal		P-Value	Postmenopausal		P-Value	P-value Between Patients Pre & Post
	Patients (n=50)	Healthy (n=40)		Patients (n=50)	Healthy (n=40)		
	Mean ± SD			Mean ±SD			
T-Ch(mg/dl)	178.30±29.89	148.89±9.51	0.001	202.75±26.82	158.98±12.28	<0.001	0.001
TG (mg/dl)	110.93±28.23	71.27±20.11	<0.001	114.15±19.65	81.50±19.49	0.001	0.628
AST(U/L)	27.68±7.06	20.25±4.19	0.001	22.75±7.22	20.03±5.73	0.294	0.007

ALT(U/L)	30.12±6.84	19.68±5.91	<0.001	25.25±8.82	17.29±6.85	0.006	0.014
ALP (U/L)	107.93±26.10	93.83±9.38	0.065	115.51±26.21	97.29±8.64	0.046	0.227
TSB (mg/dl)	0.74±0.21	0.57±0.15	0.013	0.70±0.22	0.65±0.19	0.480	0.533
DSB (mg/dl)	0.32±0.16	0.19±0.03	0.006	0.33±0.14	0.23±0.03	0.068	0.693
NSB (mg/d)	0.41±0.18	0.37±0.13	0.485	0.36±0.21	0.41±0.19	0.553	0.324

Significant difference (P≤0.05)

Inflammatory and Oxidative Stress Markers

Levels of C-reactive protein (CRP) were markedly higher in both groups of patients and healthy women. No significant differences in fibrinogen values were observed between premenopausal and postmenopausal patients, indicating that the inflammatory response in gallstone disease is similar. Table 2

Interleukin-6 (IL-6) was also enhanced in both groups of patients, but postmenopausal patients presented with significantly higher IL-6 levels (27.58±10.28 pg/mL) compared to

premenopausal patients (22.54±5.74 pg/mL). This constitutes evidence for the role of estrogen deficiency in initiating systemic inflammation. Table 2

The malondialdehyde (MDA), as an oxidative stress marker, was increased significantly in patients in the two groups, in comparison with the control group. Notably, MDA levels were higher among premenopausal than postmenopausal patients, indicating ongoing oxidative injury in both stages. Table 2

Table 2: Comparison of inflammatory parameters between patients and healthy women in premenopausal and postmenopausal age stages

Parameters	premenopausal		P-Value	Postmenopausal		P-Value	P-value between Patients Pre & post
	Patients (n=50)	Healthy (n=40)		Patients (n=50)	Healthy (n=40)		
	Mean ±SD			Mean ±SD			
CRP (pg/ml)	5.36±2.38	1.58±1.03	<0.001	5.93±2.52	1.79±0.85	<0.001	0.332
IL6 (pg/ml)	22.54±5.74	17.51±6.17	0.031	27.58±10.28	21.43±5.47	0.027	0.009
MDA (ng/ml)	287.38±29.91	186.35±27.01	<0.001	243.21±28.93	186.41±21.82	<0.001	<0.001

Gallstone Types

The results show differences in the distribution of gallbladder stone types in premenopausal and postmenopausal women. The most common type of gallstone in both the premenopausal and postmenopausal women was cholesterol stones, which accounted for 44% and 52%, respectively.

The percentage of pigment stones was higher in postmenopausal women (24%) than in premenopausal women (12%). Mixed stones were more common in premenopausal women (44%) than in postmenopausal women (24%). Table 3.

Table 3: Percentages of Gallstone Types in Premenopausal and Postmenopausal Women

Parameter	Cholesterol stone	Pigment stone	Mixed stone
Premenopausal (n=50)	22 (44%)	6 (12 %)	22 (44%)
Postmenopausal (n=50)	26 (52%)	12 (24%)	12 (24%)

Significant difference (P≤0.05)

Chemical Composition of Gallstones

Postmenopausal patients had significantly higher cholesterol content in their stones (P = 0.036) and significantly higher triglyceride levels (P = 0.001). Magnesium concentration was also

higher in this group (P = 0.035). No significant differences were observed in calcium, sodium, or potassium levels between the two groups. Table 4.

Table 4: Comparison of the composition of gallstones between patients and healthy women in premenopausal and postmenopausal age stages

Parameters	Premenopausal (n)=50 Mean ±SD	Postmenopausal(n)=50 Mean ±SD	P-Value
To-Ch (mg/dL)	165.96±27.45	181.91±27.22	0.036
TG (mg/dL)	35.10±15.99	53.84±20.18	0.001
Total bilirubin(mg/dL)	0.63±0.39	0.55±0.46	0.503
Mg(mg/dL)	2.66±0.84	3.19±0.91	0.035
Ca(mg/dL)	9.95±1.67	9.37±1.92	0.250
Na (mEq/l)	91.73±24.96	104.62±27.09	0.078
K2 (mEq/l)	6.82±1.66	7.65±1.49	0.054

Significant difference ($P \leq 0.05$)

Histopathological Changes

All patients had chronic inflammatory changes on gallbladder tissue, which included papillary hyperplasia, lymphoplasmacytic infiltration, Rokitansky–Aschoff sinuses, haemorrhage,

necrosis, and fibrinoid deposition. These pathologic features were more pronounced and widespread in postmenopausal patients, so the hormonal alterations seem to act on the gallbladder wall to increase damage.

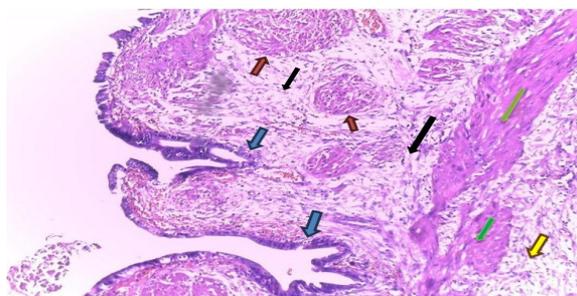


Fig 1: Histological features of the gallbladder wall with chronic cholecystitis (fundus) region showed lymphoplasmacytic type, mucosa with papillary hyperplasia (blue arrow), heavy infiltration of inflammatory cells (black arrow) extending to muscularis externa (green arrow) and adventitia (yellow arrow), and lamina propria showed granuloma (red arrow). Stain (H & E), (10X)

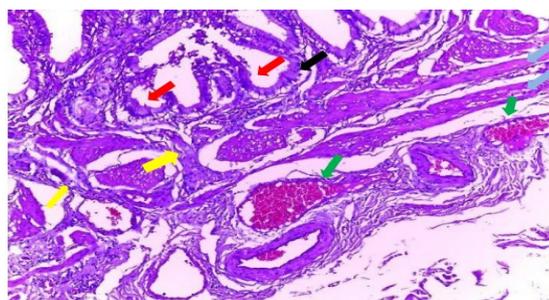


Fig 2: Histological features of the gallbladder wall with chronic cholecystitis showed Rokitansky-Aschoff sinus (blue arrow) lined with simple columnar epithelium (red arrow), haemorrhage (green arrow), hypertrophy of the muscle layer (cyan arrow), and a heavy strand of muscle fibres deposit (yellow arrow). Stain (H&E), (10X).

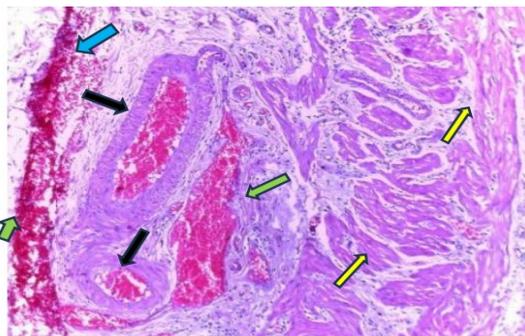


Fig 3: Histological features of the gallbladder wall with cholecystitis showed muscular hypertrophy (→), severe haemorrhage (→), and congested blood vessels (→), inflammatory response extended to the serosa region (→).(H&E) stain, (10X).

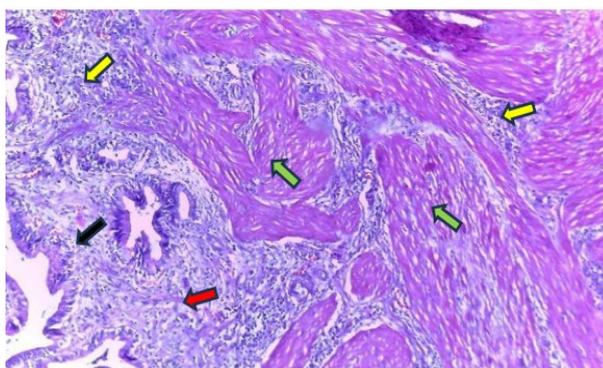


Fig 4: Histological features of the gallbladder wall with cholelithiasis showed part of the mucosa (→), loose lamina propria (→), within inflammatory cells (→) hypertrophy muscle cells (muscularis externa) (→) (H&E) stain, (40X)

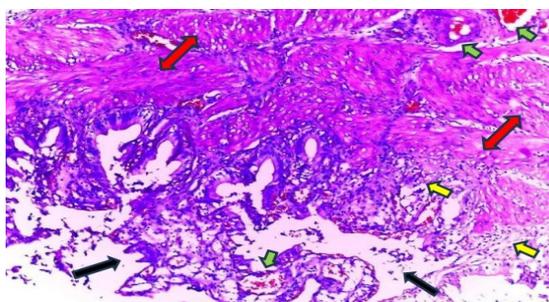


Fig 5: Histological features of the gallbladder wall with cholelithiasis showed mucosal destruction (→) , severe inflammation (→), congested blood vessels (→), necrosis and fibrin deposit (→) within the muscularis externa. (H&E)

Discussion

Histological and Biochemical Alterations in Gallbladders with Cholesterol Gallstones

Biochemical Alterations and Lipid Metabolism

The present study indicates that GSD is closely related to dyslipidaemia in pre- and postmenopausal female patients. Total cholesterol (T-Ch) and triglycerides (TG) levels were significantly higher in post menopause patients, highlighting the sex hormone-dependent role of oestrogen in lipid metabolism modulation. Oestrogen also controls hepatic LDL receptor expression and stimulates bile acid synthesis, and its decrease in post menopause

may induce bile cholesterol supersaturation and gallstone formation (3, 14, 15).

HDL cholesterol levels were lower in premenopausal patients compared to control subjects. The lower HDL in premenopausal patients likely represents an early defect in reverse cholesterol transport (RCT) (16). Oestrogen enhance HDL through stimulation of apolipoproteins and inhibition of hepatic lipase. Having higher HDL levels has been generally associated with less cardiovascular risk. Indeed, postmenopausal women have lower HDL associated with ageing and decreased estrogen, but the absence of a significant between-groups

difference suggests an ageing-related decline in both patients and controls (17).

Increased triglycerides in both hormonal groups point to oestrogen-independent metabolic alterations linked to lithogenicity, resulting in gallbladder hypomotility, increased hepatic VLDL production and changes in bile composition towards a more lithogenic environment (18, 19, 20).

Liver Enzymes and Hepatic Dysfunction

Liver enzymes were elevated in gallstone patients, particularly ALT in premenopausal patients, pointing to hepatocellular stress from lipid accumulation or inflammation. AST levels were slightly lower in postmenopausal women, which may reflect reduced muscle contribution or an impaired inflammatory response (21). ALP increasing in postmenopausal women may also stem from oestrogen deficiency-related bone resorption rather than hepatobiliary dysfunction (22). Similar results were reported internationally, ensuring the tissue-specific effects of menopause on hepatic biochemistry (15, 23).

In this analysis, AST levels seemed to decrease in postmenopausal patients versus premenopausal women, potentially reflecting lower release of extra-hepatic AST, observed when there is less underlying muscle mass, or a weakened inflammatory state resulting from the withdrawal of oestrogen (15, 23).

DSB was elevated in premenopausal patients compared to controls, which suggests additional modulators beyond oestrogen, with partial adaptation mechanisms in postmenopausal patients with gallstones (24, 25). whereas NSB remained stable across all groups, agreement with global data that display limited hormonal influence on heme catabolism or hepatic uptake transporters (26).

Inflammatory and Oxidative Stress Markers

CRP and IL-6 were significantly increased in both hormonal groups, ensuring systemic inflammation in GSD. The stronger IL-6 response in postmenopausal patients supports the anti-inflammatory role of oestrogen, which downregulates IL-6 by NF- κ B inhibition (27, 17).

Oxidative stress, as determined by MDA, was significantly higher among gallstone patients in both groups, and postmenopausal patients had the highest values. This result further emphasises an antioxidative role of the oestrogen, and suggests that oxidative injury is an important etiological factor in the pathogenesis of gallstone disease (28, 29).

MDA were significantly elevated in all patient groups, with postmenopausal women showing the highest levels, enhancing the antioxidant role of oestrogen and the central role of oxidative

stress to gallstone pathogenesis (28, 29). Chronic gallbladder inflammation promotes IL-6 and CRP release, downregulating CCKAR and decreasing motility, ultimately leading to stone formation (30, 31). Elevated oxidative stress in premenopausal patients may reflect higher metabolic demand, consistent with reports linking lipid turnover to oxidative injury (32, 33). The combined rise of CRP, IL-6, and MDA confirms oxidative and inflammatory injury as a hallmark of biliary dysfunction (34).

Gallstone Composition and Mineral Content

Cholesterol stones dominated in both hormonal groups, particularly in postmenopausal patients, showing greater triglyceride and magnesium content. Pigment stones were more frequent in post menopause, consistent with ageing-related stasis and oxidative damage (34). Triglyceride-rich stones exhibiting lower crystallinity and higher amorphous structure, as assured by spectroscopic research suggesting hormonal effect on stone microstructure (35, 36).

Magnesium level in gallstones of premenopausal women is usually rather equilibrated and included in the bile matrix, being frequently associated with phospholipids and bile salts to preserve the fluidity of the bile (37, 38). The findings indicate that the incorporation of calcium into gallstones is not influenced very much by the menopausal status, supporting reports that systemic oestrogen-dependent calcium changes do not affect biliary calcium (38). Potassium and sodium values in stones were higher in postmenopausal women but not significantly, pointing to stability across reproductive stages (39, 40, 41). Total bilirubin value did not differ between groups, suggesting stable pigment metabolism despite hormonal transition (34).

Histological Changes of the Gallbladder

Histological results were more severe in postmenopausal women, with papillary hyperplasia, lymphoplasmacytic infiltration, Rokitansky-Aschoff sinuses, muscular hypertrophy, haemorrhage, and necrosis. These findings refer to chronic inflammation and oestrogen-decrease-driven remodelling (42). Differentiation from early neoplastic lesions remains important, as irregular glandular structures and atypia may mimic carcinoma (43, 44, 45). Granulomatous reactions with foamy macrophages were also observed, proportionate with chronic irritation by bile salts or cholesterol crystals (46).

Vascular injury was dominant, including haemorrhage, fibrin deposition, and small-vessel thrombosis, particularly near the fundus, which is prone to ischemic injury (47). Chronic immune-mediated granulomas and fibrinoid deposits

highlight the risk of transmural involvement, complications such as fibrosis or perforation, and favour the necessity of histopathological stimulation in GSD management.

Study Limitations

This study is limited by its single-centre design, cross-sectional nature, and the lack of direct hormonal measurements (e.g., oestrogen, progesterone). These constraints limit causal interpretation and generalizability. Future multicentre longitudinal studies with integrated hormonal profiling are recommended to clarify the temporal and mechanistic links between menopause, metabolic dysregulation, and gallstone disease.

Conclusion

In conclusion, the study indicates a strong relationship between menopausal state and biochemical and histopathologic disturbances in GSD. They have a more marked increase in inflammatory and oxidative markers and in damage to the gallbladder wall, and this could be due to oestrogen deficiency. The findings highlight the importance of hormone status in the context of gallstone disease management and provide a rationale for further studies focusing on personalised treatment approaches involving inflammation and oxidative stress pathways in postmenopausal cohorts.

List of abbreviations

ALP: Alkaline Phosphatase
ALT: Alanine Aminotransferase
AST: Aspartate Aminotransferase
CRP: C-reactive protein
FBG: Fasting blood glucose
GSD: Gallstone disease
HDL: High-density lipoprotein
IL-6: Interleukin-6
LDL: Low-density lipoprotein
MDA: Malondialdehyde
PCT: Procalcitonin
T-Ch: Total cholesterol
TG: Triglycerides
VLDL: Very low-density lipoprotein-cholesterol

Declaration

Ethical Considerations

This study was approved by the Ethics Committee of the Ministry of Health, Iraq, under approval number (3428. Date: 2023/10/18. Written informed consent was obtained from all participants. Data confidentiality and participant anonymity were strictly maintained. The study complied with the principles of the Declaration of Helsinki. Before recruitment, participants were fully informed about the objectives and processes of the study, and written consent was obtained.

Novelty Statement

This study uniquely correlates biochemical, inflammatory, and histological changes in gallstone disease among pre- and postmenopausal women. It highlights how menopausal status distinctly affects lipid metabolism, inflammatory markers, and gallbladder structure, revealing novel stage-specific patterns. These findings support the development of personalised diagnostic and treatment strategies based on hormonal status.

Author's Contribution

The author was solely responsible for the study design, data collection, analysis, interpretation, and manuscript writing

Source Of Support

There is no source of funding or support for this study from any governmental or private entity.

Conflict Of Interest

The researchers declare that there are no conflicts of interest regarding this manuscript. The style of writing and subject matter of the article are the sole responsibility of the authors.

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