ORIGINAL ARTICLE

Oxidative Stress and Phospholipid Alterations in Destructive Cholecystitis and Cholelithiasis: A Comprehensive Analysis

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Abstract

Background: Oxidative stress, lipid peroxidation (LPO), and antioxidant depletion are crucial in the pathogenesis of destructive cholecystitis and cholelithiasis. **Objectives:** This study investigated the influence of these factors on the phospholipid (PL) composition of erythrocyte membranes in patients with acute calculous cholecystitis (ACC), chronic calculous cholecystitis, and gangrenous ACC. **Methods:** Markers of LPO, antioxidant defense, and endogenous intoxication were measured in blood plasma and erythrocyte membranes. The PL fractions in the erythrocyte membranes were analyzed using thin-layer chromatography. **Results:** The results showed increased levels of diene conjugates and malondialdehyde, indicating significant LPO in destructive cholecystitis cases. Antioxidant defenses were depleted, as evidenced by the decreased total antioxidant activity and catalase levels. Elevated levels of medium-molecular-weight peptides and phospholipase activity indicate systemic inflammatory responses and internal intoxication. Notable changes in the erythrocyte membrane PL composition included increased lysophosphatidylcholine and reduced phosphatidylcholine levels, suggesting membrane degradation. The increased ratio of easily oxidizable PL fractions to less oxidizable fractions indicates membrane instability and oxidative stress. **Conclusion:** These findings confirm that oxidative stress, PL changes, and weakened antioxidant defense contribute to the progression of destructive cholecystitis and cholelithiasis. Understanding these molecular alterations can help identify novel diagnostic markers and therapeutic targets for biliary tract diseases.

Key words: Cholelithiasis, destructive cholecystitisoxidative stress, erythrocyte membranes, lipid peroxidation, phospholipids

INTRODUCTION

Cholelithiasis and destructive cholecystitis are major causes of gastrointestinal surgical illness. Despite advances in imaging and surgery, their cellular mechanisms are unclear. Evidence indicates that oxidative stress, lipid peroxidation (LPO), and reduced antioxidant defenses play key roles in gallbladder (GB) inflammation and gallstone formation through their effects on biological membranes.[1,2]

The mucosal lining of the GB and hepatobiliary system is vulnerable to reactive oxygen species, which trigger LPO. This process damages cell membrane stability by targeting fatty acids in phospholipids (PLs), forming harmful byproducts such as diene conjugates (DC) and malondialdehyde (MDA).[3,4] These substances trigger inflammatory responses and cellular malfunctions, causing oxidative harm.

Numerous studies have explored the issues of acute cholecystitis and cholelithiasis, focusing on diagnosis, pathogenesis, treatment strategies, and prevention of

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complications.[1,5] Despite this extensive research, the cellular mechanisms underlying the pathophysiological changes during stone formation and GB tissue inflammation, as well as the progression from catarrhal to purulent inflammatory processes, are not yet fully understood. From a pathophysiological perspective, the development of destructive cholecystitis in patients with cholelithiasis involves a complex mechanism. This includes the processes of free-radical LPO and the antioxidative system (AOS), which are crucial because of their role in the cellular mechanisms of inflammation affecting organs and systems in the human body.

The AOS, comprising enzymatic and non-enzymatic components, is often overwhelmed in DC and acute cholecystitis, allowing oxidative imbalance.[2,6] This redox imbalance intensifies inflammation and disrupts membrane functions, including ion transport and signal transduction.[7] Medium-molecular-weight peptides (MMPs), generated during inflammation, display pathophysiological activity by imitating regulatory peptides and interacting with membrane receptors, indicating a systemic inflammatory response.

The activation of proteolysis forms MMP with molecular weights ranging from 300 to 5000 daltons, which impact organ function due to their similarity to regulatory peptides. MMP can bind to cell receptors, affecting metabolism and function. An imbalance between free radical LPO and antioxidant defense, along with endogenous intoxication, causes oxidative stress that disrupts cell membrane structures, particularly their PL composition.[4,7]

Erythrocyte membranes, despite lacking nuclei and organelles, reflect physiological changes and oxidative status.[8] Changes in membrane PLs, including decreased phosphatidylcholine (PC) and increased lysophosphatidylcholine (LPC) levels, indicate oxidative stress and phospholipase activation.[9]

Although insights have been gained, specific changes in PLs within erythrocyte membranes during destructive cholecystitis and cholelithiasis remain poorly understood. Investigating this area could improve understanding of membrane pathology in biliary diseases and identify potential biomarkers for assessing disease severity. This study evaluated the influence of oxidative stress, endogenous intoxication, and antioxidant defenses on the PL composition of erythrocyte membranes in patients with destructive cholecystitis and cholelithiasis.

MATERIALS AND METHODS

This study included 324 patients diagnosed with destructive cholecystitis and cholelithiasis from May 2021 to December 2024. Among them, 197 had acute calculous cholecystitis (ACC), 97 had chronic CC (CCC), and 30 had gangrenous ACC (GACC). Confirmation of destructive cholecystitis was achieved through surgical procedures using both laparotomic and laparoscopic techniques. For comparison, 38 healthy adults were included as the control group. This study was conducted with informed consent from the patients and was approved by the Bioethics Committee of the International Higher School of Medicine (Protocol No. 11, dated December 18, 2020).

Women comprised 81.2% of the patients. Regarding age, 71.4% of patients were >44 years old. Most patients (79.3%) sought medical attention 24 h after disease onset. Laparotomic surgery was performed in 81% of the patients. Blood plasma and erythrocyte membranes were used as substrates in specialized studies. Erythrocyte membranes mirror the properties of cytoplasmic membranes, making them a useful model for examining biomembrane properties in physiological and pathological contexts.

The membranes of erythrocytes were isolated after removing the heparinized plasma and washing with a physiological solution. Erythrocytes were separated by centrifugation at 1500 rpm for 20 min. Hemolysis was performed in a 20-fold volume of 0.005 moL/L sodium phosphate buffer at pH 8.0. The erythrocyte shadows were sedimented at 3000 rpm for 30 min and washed twice with hemolysate medium and once with buffer solution at pH 7.4. Both primary and secondary LPO products were measured to evaluate oxidative stress markers in erythrocyte membranes. LPO was evaluated by measuring the DC concentration in the erythrocyte membranes. This method relies on the ultraviolet absorption of lipid extracts from erythrocyte shadows. MDA levels in erythrocyte membranes were determined using a biochemical method with thiobarbituric acid, and results were expressed in units of optical density per mL of membrane suspension.

Antioxidant defense was evaluated by measuring the total antioxidant activity (AOA) of the blood plasma. This assessment was based on AOA's ability to inhibit free radical oxidation of erythrocyte shadows, where oxidation is triggered by ultraviolet light, and catalase activity in blood plasma, which relies on hydrogen peroxide's capacity to form a stable colored complex with ammonium molybdate salts. Endogenous intoxication was evaluated by measuring MMPs concentration in blood and phospholipase activity. The MMPs concentration in blood plasma was determined using a spectrophotometric method. Phospholipase activity in erythrocytes was assessed through toxic hemolysis of red blood cells during incubation with a standard lecithin solution, expressed as a percentage of erythrocyte hemolysis.

The PL fractions in the erythrocyte membranes were analyzed using thin-layer chromatography on Silufol plates. The identified PL fractions included LPC, sphingomyelin (SM), PC, phosphatidylserine (PS), and phosphatidylethanolamine (PEA). The PL quantity was assessed by measuring the phosphorus content in each fraction. This method uses the reaction between inorganic phosphorus and ammonium

molybdate to form phosphomolybdic acid, which is reduced by ascorbic acid to produce colored molybdate oxides. The PL fraction content was expressed as a percentage of the total PL.

All assessments of biochemical and membrane composition were conducted thrice to ensure data reliability and consistency. Statistical analyses were performed using Statistica v8.0 (StatSoft, Inc., Tulsa, OK, USA). Continuous data were presented as mean ± standard deviation and were analyzed using the independent *t*-test. Categorical data were presented as frequencies and percentages. Statistical significance was assessed as follows: differences between patient groups were deemed significant at $P < 0.05$ (*), while differences between patients and controls were significant at $P < 0.01$ (**). Differences were considered highly significant at $P < 0.001$ (***).

RESULTS

The increased DC and MDA levels in the clinical groups indicate significant LPO in destructive cholecystitis. These substances are formed through the oxidative breakdown of membrane polyunsaturated fatty acids, compromising membrane integrity. The marked decrease in total antioxidant and catalase activities indicates depleted antioxidant defenses. This redox imbalance creates a proinflammatory environment, leading to tissue damage and progression from catarrhal to gangrenous inflammation.

According to this study (Table 1), there was an increase in the levels of initial (DC) and final products (MDA) of LPO in phlegmonous and gangrenous GB inflammation upon admission compared to healthy individuals ($P < 0.001$). However, the values within the clinical groups showed no significant differences. During this examination period, the AOS indicators revealed a reduction in the total AOA of blood plasma and catalase activity ($P < 0.01$–$P < 0.001$). In addition, significant changes were observed in endogenous intoxication indicators, measured by MMPs concentration in blood plasma and endogenous phospholipase activity in erythrocytes.

High levels of MMPs and phospholipase activity in the patient groups indicated systemic inflammatory responses and internal intoxication. MMPs accumulate during protein breakdown and signal cellular degradation and inflammation. Increased phospholipase activity indicates membrane PL breakdown and the production of pro-inflammatory lipid mediators, such as LPC. These findings suggest that systemic membrane destabilization indicates broader pathophysiological changes beyond local GB issues.

The concentration of MMPs in destructive forms of cholecystitis was higher than in individuals from the control group ($P < 0.01$), and phospholipase activity increased by 2.5 times ($P < 0.01$). Meanwhile, the indicators between the clinical groups showed no significant differences.

Alterations in erythrocyte membrane PL composition validate the oxidative and enzymatic damage. An increase in LPC and a decrease in PC indicate increased phospholipase activity and disrupted membrane biosynthesis. The heightened (PS+PEA)/(SM+PC) ratio in all patient groups suggests a shift toward more oxidizable PL fractions, indicating membrane instability and oxidative stress. This PL restructuring reflects systemic inflammation and membrane damage in destructive cholecystitis.

Notable changes were observed in the erythrocyte membrane PL composition (Table 2). In destructive cholecystitis, there was a twofold increase in the LPC fraction and a reduction in the primary structural component of PL-PC ($P < 0.01$). This resulted in an increase in easily oxidizable PL fractions (PS and PEA), as evident in the coefficient value representing the ratio of total easily oxidizable fractions to less oxidizable fractions ($P < 0.01$).

Due to active metabolic activity in the GB, oxidative peroxidation processes occur at elevated levels. These processes primarily occur in the cell membrane PLs, as they contain significant amounts of unsaturated fatty acids. The pathophysiological aspects of LPO are evident in the increased cell membrane permeability to various ions and molecules. If excess peroxide products are not neutralized by the AOS, further oxidative processes proceed through

Table 1: Markers of LPO, antioxidant defense, and endogenous intoxication in blood plasma and erythrocyte membranes of patients with destructive cholecystitis and cholelithiasis

Group	DC (nmoL/mg protein)	MDA (U/mL)	AOA (%)	Catalase (mkat/L)	MMPs (Ed)	Phospholipase activity (% hemolysis)
ACC	1.86±0.07***	2.04±0.09***	11.2±0.95***	13.1±0.96**	0.31±0.03**	21.5±1.4**
CCC	1.62±0.07***	1.98±0.09***	12.6±0.94***	14.2±0.98**	0.29±0.04**	19.6±1.3**
GACC	2.02±0.09***	2.30±0.07***	9.4±0.76***	11.5±0.82***	0.361±0.05**	24.4±1.7**
Control	0.64±0.03	0.84±0.03	24.6±1.4	22.1±1.5	0.20±0.03	9.4±0.74

ACC: Acute calculous cholecystitis, CCC: Chronic calculous cholecystitis, GACC: Gangrenous acute calculous cholecystitis, DC: Diene conjugates, MDA: Malondialdehyde, AOA: antioxidant activity, MMPs: Medium-molecular-weight peptides. Values are expressed as mean±standard deviation. *P<0.05, **P<0.01, ***P<0.001

Table 2: Phospholipid composition of erythrocyte membranes in patients with destructive cholecystitis and cholelithiasis

Group	LPC (%)	SM (%)	PC (%)	PS (%)	PEA (%)	(PS+PEA)/(SM+PC)
ACC	15.4±0.76**	18.1±0.98	20.2±1.32**	21.5±1.41	25.2±1.71	1.34±0.03**
CCC	14.6±0.83**	19.0±1.2	18.4±1.31**	23.1±1.4	26.6±1.65	1.34±0.04**
GACC	17.5±0.94**	17.9±1.1	19.1±1.44**	22.0±1.51	26.2±1.83	1.38±0.04**
Control	7.4±0.39	19.6±1.0	28.4±1.72	19.4±1.31	23.6±1.5	0.90±0.03

LPC: Lysophosphatidylcholine, SM: Sphingomyelin, PC: Phosphatidylcholine, PS: Phosphatidylserine, PEA: Phosphatidylethanolamine. Values are expressed as mean±standard deviation. *P<0.05, **P<0.01, ***P<0.001

chain mechanisms involving the initiation, continuation, and branching of chain reactions.

MMP levels in the bloodstream indicate internal intoxication due to inflammation and pus formation in the GB. LPO occurs in response to pathological conditions. However, the peroxidation activity and AOA degree can reveal both the nature of inflammation and the extent of organ damage. A significant buildup of peroxide products within cells can alter biological membrane function by influencing cell membrane enzymes, such as RNase, succinate dehydrogenase, and acetylcholinesterase, primarily due to PL structural damage. Increased LPO affects inflammation and aids in GB stone formation.

An increase in LPO fraction signals pathology "markers" at the cellular level, causing violations of membrane permeability, transport, fluidity, stability, and cell shape changes. The depth of membrane damage in destructive cholecystitis and cholelithiasis is indicated by a decreased PC fraction. Lower PC levels in membrane complexes indicate a reduced antioxidant role in cellular structures due to free radical lipid oxidation products. The decrease in PC concentration also reflects the activation of endogenous phospholipase, the main enzyme that breaks down PLs. A relative increase in easily oxidizable PL fractions (PS and PEA) may indicate a higher PL exchange intensity in cell membranes, with unsaturated fatty acids predominating. These fractions participate in cell membrane permeability and ion transport processes. An increased PL coefficient indicates higher cell membrane permeability and signals adaptation to tension in destructive cholecystitis.

DISCUSSION

This study demonstrates the influence of oxidative stress, depletion of antioxidants, and PL remodeling in the development of destructive cholecystitis and cholelithiasis. Our results validate that an imbalance between LPO and antioxidant defense systems causes GB inflammation, consistent with previous studies on tissue damage induced by reactive oxygen species.[10,11]

In patients with ACC, CCC, and GACC, the levels of DC and MDA, which are indicators of primary and secondary LPO, were increased. These findings align with research showing oxidative stress in GB tissues during cholecystitis and its role in membrane lipid breakdown.[12,13] MDA, the end product of polyunsaturated fatty acid oxidation, is a reliable marker of membrane damage and inflammation.[14]

The decrease in catalase and AOA levels across patient groups indicates depletion of the enzymatic antioxidant system, which neutralizes hydrogen peroxide and prevents oxidative chain reactions. These results are consistent with those of Li *et al*., who found that compromised antioxidant enzyme activity in hepatobiliary diseases exacerbates oxidative membrane damage.[15]

This study examined the erythrocyte membrane PL composition and showed an increase in LPC and a reduction in PC across the different types of cholecystitis. These findings align with those of studies showing that LPC buildup from phospholipase A2 activation causes inflammation.[16] This study found elevated ratios of oxidizable PL fractions, PEA, and PS compared to SM and PC. This modified (PS+PEA)/(SM+PC) ratio indicates redox imbalance and membrane susceptibility.[17] Elevated MMPs and phospholipase activity indicate systemic inflammation, tissue breakdown, and membrane damage.[18]

Bekov *et al*. corroborated these results by showing that patients with cholecystitis have erythrocyte membranes with elevated oxidative stress markers and structural PL alterations, which align with membrane damage from inflammation.[19] In addition, Bekov *et al*.'s sonographic evaluations of GB wall thickening, vascular resistance, and volume in destructive cholecystitis reflect the pathological changes observed at the molecular level, providing a perspective on disease severity.[20]

The detected oxidative and enzymatic changes highlight the importance of redox balance in GB pathology, with erythrocyte membranes serving as indicators of systemic inflammation. These molecular markers may help assess the severity and progression of cholecystitis. Future studies should explore therapies that target oxidative stress and PL metabolism to reduce inflammation. In addition, identifying specific PLs that cause membrane instability could improve early diagnosis and monitoring.

CONCLUSION

This study demonstrates that oxidative stress, PL changes, and weakened antioxidant defenses play crucial roles in destructive cholecystitis and cholelithiasis. Increased LPO indicators, such as DC and MDA, with decreased total AOA and catalase levels, indicate redox imbalance and damage to the GB tissue. An increase in MMPs and phospholipase activity indicates systemic inflammation. Analysis of erythrocyte membrane PLs revealed increased LPC and reduced PC levels, indicating membrane degradation.

The shift in the (PS+PEA)/(SM+PC) ratio suggests increased membrane susceptibility to oxidative harm. These findings confirm that LPO and antioxidant depletion are major contributors to the progression from catarrhal to gangrenous cholecystitis. Understanding these molecular changes can help identify novel diagnostic markers and therapeutic targets. Future studies should focus on restoring redox balance and membrane stability to reduce the severity of biliary tract diseases.

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REFERENCES

1. Kiriyama S, Takada T, Strasberg SM, Solomkim JS, Mayumi T, Pit HA, *et al*. New diagnostic criteria and severity assessment of acute cholangitis in revised Tokyo guidelines. J Hepatobiliary Pancreat Sci 2012;19:548-56.
2. Terekhina NA, Zarivchanskiĭ MF, Vladimirov AA, Khlebnikov VV. Antioxidative defense parameters in acute and chronic cholecystitis. Klin Lab Diagn 2008;4:41-3.
3. Cimato A, Facorro G, Aguirre F, Hager A, De Paoli, Ihlo J, *et al.* A spectrophotometric method for the determination of hydroperoxides in liposomes. Spectrochim Acta A Mol Biomol Spectrosc 1998;54A:2001-8.
4. Grintzalis K, Papapostolou I, Assimakopoulos SF, Mavrakis A, Faropoulos K, Karageorgos N, *et al.* Time-related alterations of superoxide radical levels in diverse organs of bile duct-ligated rats. Free Radic Res 2009;43:803-8.
5. González-Castillo AM, Sancho-Insenser J, De Miguel-Palacio M, Morera-Casaponsa JR, Membrilla-Fernández E, Pons-Fragero MJ, *et al.* Mortality risk estimation in acute calculous cholecystitis: Beyond the Tokyo guidelines. World J Emerg Surg 2021;16:24.
6. Alho H, Leinonen JS, Erhola M, Lönnrot K, Aejmelaeus R. Assay of antioxidant capacity of human plasma and CSF in aging and disease. Restor Neurol Neurosci 1998;12:159-65.
7. Karadeniz G, Acikgoz S, Tekin IO, Tascýlar O, Gun BD, Cömert M. Oxidized low-density-lipoprotein accumulation is associated with liver fibrosis in experimental cholestasis. Clinics (Sao Paulo) 2008;63:531-40.
8. Lehtinen M, Koivisto V, Lahtinen P, Lehtinen T, Aaran RK, Leinikki P. Phospholipase A2 activity is copurified together with herpes simplex virus-specified Fc receptor proteins. Intervirology 1988;29:50-6.
9. Jalilov A, Uralov SM, Achilova FA. The state of the erythron system in acute pneumonia in children. Web Sci 2022;3:798-808.
10. Shaffer EA. Gallstone disease: Epidemiology of gallbladder stone disease. Best Pract Res Clin Gastroenterol 2006;20:981-96.
11. Reuter S, Gupta SC, Chaturvedi MM, Aggarwal BB. Oxidative stress, inflammation, and cancer: How are they linked? Free Radic Biol Med 2010;49:1603-16.
12. Konopacka M. Role of vitamin C in oxidative DNA damage. Postepy Hig Med Dosw (Online) 2004;58:343-8.
13. Gutteridge JM. Lipid peroxidation and antioxidants as biomarkers of tissue damage. Clin Chem 1995;41:1819-28.
14. Esterbauer H, Schaur RJ, Zollner H. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. Free Radic Biol Med 1991;11:81-128.
15. Li S, Tan HY, Wang N, Zhang ZJ, Lao L, Wong CW, *et al.* The role of oxidative stress and antioxidants in liver diseases. Int J Mol Sci 2015;16:26087-124.
16. Dennis EA, Norris PC. Eicosanoid storm in infection and inflammation. Nat Rev Immunol 2015;15:511-23.
17. Berliner JA, Leitinger N, Tsimikas S. The role of oxidized phospholipids in atherosclerosis. J Lipid Res 2009;50 Suppl: S207-12.
18. Tabet F, Rye KA. High-density lipoproteins, inflammation and oxidative stress. Clin Sci (Lond) 2009;116:87-98.
19. Bekov T, Atykanov A, Tagaev T, Vityala Y. Impact of medication and surgical treatment on cytokine concentrations in patients with acute and chronic cholecystitis. Asian J Pharm 2024;18:222-5.
20. Bekov T, Mamasaliev J, Baigashkaev E, Tagaev T. Ultrasound diagnosis of cholecystitis and cholelithiasis: A study of various types and complications for better management. Asian J Pharm 2024;18:968-71.

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