Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 16s (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

Obesity- Associated Gallstone Complications: Investigating HMG-CoA Gene Expression and Spexin Biomarkers

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Cite this paper as: Rusul Ayar Kassim, Nawal Khinteel Jabbar, (2025) Obesity- Associated Gallstone Complications: Investigating HMG-CoA Gene Expression and Spexin Biomarkers. *Journal of Neonatal Surgery*, 14 (16s), 87-95.

ABSTRACT

Gallstone disease (GSD) is a prevalent and painful disorder characterized by the development of solid deposits in the gallbladder or bile ducts, including cholesterol, bile pigments, and calcium. While obesity is the main risk factor for GSD, the condition can also affect individuals who are not obese. This study examined the factors in obese and non-obese patients that may contribute to the formation and progression of gallstones, emphasizing the roles of β-Hydroxy β-Methylglutaryl Co-A gene expression and Spexin levels in driving inflammation.

The study involved 120 participants: 30 patients with symptomatic gallstones and obesity, 30 patients with symptomatic gallstones but no obesity, 30 obese patients without gallstones, and 30 healthy controls. Lipid profile levels (total cholesterol, triglycerides, HDL, LDL, and VLDL) were measured using an automatic Fuji film analyzer. Serum C-reactive protein (CRP) was quantified using an I-Chroma reader. Serum SPX levels were determined by enzyme-linked immunosorbent assay (ELISA), and HMG Co-A gene expression was analyzed using quantitative polymerase chain reaction (qPCR). Data were analyzed with SPSS version 28, with statistical significance set at $P < 0.05$.

The findings indicated an increase in lipid profile values and a decrease in HDL levels in all patient groups compared to controls, which was associated with heightened inflammation, as reflected by elevated CRP levels. Additionally, there were decreased SPX levels and a rise in HMG Co-A gene expression ($P < 0.05$).

In conclusion, the study suggests that the levels of SPX are negatively correlated with HMG Co-A gene expression, which may play a crucial role in triggering inflammation in gallstone patients, thus contributing to the progression of the disease, particularly in obese individuals.

Keywords: *Gallstone, Obesity, β-Hydroxyl β-Methyl glut aryl Co-A, Spexin, C-reactive protein, Lipid Profile.*

1. INTRODUCTION

Gallstone disease is considered one of the most excruciating conditions in adults and is highly prevalent across global populations [1]. Gallstones are hard formations of bile salts that develop in the gallbladder or bile ducts. They comprise cholesterol, bile pigments, and calcium ions [2].

The primary risk factors for gallstone formation include being female, pregnancy, a family history of the condition, genetic factors, obesity, type 2 diabetes (which causes neuropathy and decreases gallbladder contractility), estrogen [3], elevated triglyceride levels, hyperinsulinemia, low levels of high-density lipoprotein, and diets high in carbohydrates and fats [4].

The majority of gallstones remain asymptomatic and do not cause any noticeable symptoms, typically being detected incidentally during an abdominal ultrasound (US) [5]. Common symptoms of symptomatic gallstones include intense, persistent pain in the right upper abdomen, along with nausea, vomiting, fever, and jaundice [6].

Obesity is defined as a chronic, low-grade inflammatory state that leads to metabolic alterations as a result of excessive fat accumulation [7]. Obesity occurs when there is an abnormal or excessive accumulation of fat in adipose tissue, which can negatively impact health [8]. This condition has been linked to a range of medical problems, including gallbladder disease. Therefore, understanding the relationship between obesity and gallstone disease is crucial for both preventing and effectively treating these conditions [9].

Gallstones can lead to inflammation of the gallbladder, which may result in acute or chronic cholecystitis [1]. Although both types of inflammation are crucial to the pathophysiology of gallbladder disease, there has been limited attention on the role of inflammatory markers in diagnosing or assessing the extent of tissue damage. C-reactive protein (CRP) has gained

recognition as a key marker for evaluating disease activity in gallstone-related conditions. A retrospective study demonstrated a strong correlation between CRP levels and the severity of acute cholecystitis, indicating that CRP is valuable not only for diagnosing the disease but also for tracking its progression [10].

Spexin (SPX), also known as neuropeptide Q (NPQ), was recently discovered, SPX is a 14-amino acid neuropeptide that is highly conserved across mammalian and non-mammalian species [11]. SPX is produced in various tissues, including white adipose tissue, the brain, heart, thyroid, lungs, ovaries, liver, adrenal glands, testes, pancreas, muscles, and [12]. SPX is crucial in regulating bile acid metabolism. In vivo studies have shown that SPX inhibits bile acid synthesis by repressing CYP7A1, an effect that can be effectively reversed through GALR2/3 blockade. [13]. SPX is believed to have an anti-inflammatory role, which could be particularly important given that chronic inflammation plays a crucial role in the development of gallstones [14]. Although direct studies connecting SPX to gallstones are limited, its known role in obesity, inflammation, and metabolic regulation offers a basis for investigating its potential involvement in gallstone development [15]. SPX has been identified as a key regulator of obesity and its related metabolic disorders, including insulin resistance (IR) and diabetes mellitus (DM) [16]. Recent studies have highlighted the potential of SPX as an anti-obesity treatment. Mona A. Said and colleagues found that administering SPX resulted in a reduction in body weight in mice fed a high-fat diet, suggesting that SPX could be used as a therapeutic option for obesity [17].

The key enzyme involved in the synthesis of new cholesterol, β-Hydroxy β-Methylglutaryl Co-A (HMG-CoA), has been implicated in the formation of gallstones. The association between HMG-CoA activity and gallstone development suggests a potential mechanistic link in the pathogenesis of the condition [18].

This study aims to investigate the interactions among various pathways that contribute to the exacerbation of gallstones including inflammation, which enhance the expression of HMG Co-A genes. Additionally, it explores the role of the inflammatory factor SPX with these factors. The research seeks to demonstrate the impact of obesity on these pathways in the context of gallstone complications. Understanding the mechanisms and the connections between these factors could help identify potential therapeutic strategies.

2. MATERIALS AND METHODS:

Collection of Blood Samples

The study includes (120) subjects, (30) patients with symptomatic gallstones & obesity, (30) patients with symptomatic gallstones, (30) patients with obesity and (30) Controls. The mean age of the population ranged from 18 to 45 years.

Five milliliters of blood samples were collected from all study groups, one milliliter was put into K_2-EDTA tubes at (-80°C) for gene expression analysis, while the remaining four milliliters were allowed to clot for 30 minutes in gel tubes. The serum was separated from the remaining blood by centrifugation at (4000 rpm) for 15-20 minutes. The separated serum was divided into parts using Eppendorf tubes (300 µl) at (-40°C) for biochemical analysis.

Inclusion Criteria

The specialist physician identified all groups depending on BMI and Ultra-Sound examination.

Exclusion Criteria

The study excluded patients with diabetics, Hypertension, thyroid disorders, autoimmune diseases, and cancer patients.

Method

Lipid profile levels were measured using an automatic Fuji film analyzer.

CRP concentrations were automatically calculated by the i-Chroma™ reader, which provided results ranging from 2.5 to 300 mg/L. (i-Chromα™, Boditech, Korea).

The serum levels of SPX were computed using the sandwich enzyme-linked immunosorbent assay (ELISA) technique as directed by the manufacturer (ELISA Kit, Elabscience®, USA).

β-Hydroxy β-Methyl glutaryl Co-A gene expression and determined the folding in samples was measured using reverse transcription-quantitative polymerase chain reaction assay (RT-qPCR) by Livak's formula.

Primers used for qPCR experiments

(Table 1) Target gene (β-Hydroxy β-Methylglutaryl Co-A)

Sequence (5'->3'))	Template strand (5'……3')	Start	Stop
HMG Co-A -F	GCACCCCAGGGATCATGAAA	640	659
HMG Co-A -R	TACAGGTAGCCTGAGCCACT	965	984

Gene bank Accession number: L07033

(Table 2) Housekeeping gene (Glyceraldehyde 3-phosphate dehydrogenase)

Sequence (5'->3')	Template strand (5'……3')	Start	Stop
GAPDH-F	CCAGAACATCATCCCTGCC	4289	4307
GAPDH-R	AAGATGAAAAGAGTTGTCAGGGC	4696	4718

Gene bank Accession number: J04038

Statical Analysis

Version 28 of SPSS was used for statistical analysis. Shown as mean and SD. Comparison of groups with normal and non-normal distribution data using Kruskal Wallis or one-way ANOVA with Tukey post hoc analysis. A $P < 0.05$ was considered significant throughout the trial. Correlation coefficients show how two continuous variables relate.

3. RESULTS AND DISCUSSION:

This study included a total of 90 patients and 30 healthy controls. The patients' characteristics and biochemical assay data are presented in Table 3. There are non-significant differences in age and gender in all groups.

There are non-significant differences between Gallstone with obesity and obesity groups, and there are insignificant differences between Gallstone without obesity and Control subjects regarding BMI, but a significant difference between obese groups and Gallstone with obesity and control ($P < 0.05$).

(Table 3) Demographic characteristics of patients and healthy controls.

Characteristic	Gallstone with obesity n=30	Gallstone without obesity n=30	Obesity only n=30	Control subjects n=30	*P*
Age (years)					
Mean ±SD	37.13± 8.02	35.93± 8.87	34.93± 8.34	33.20± 7.23	0.187 † NS
Range	20-45 years	20-45 years	20-45 years	20-45 years	
< 30, *n* (%)	5 (16.7 %)	6 (20.0 %)	9 (30.0 %)	12 (40.0 %)	0.375 ¥ NS
30-39, *n* (%)	13 (43.3 %)	13 (43.3 %)	10 (33.3 %)	12 (40.0 %)	
≥ 40, n (%)	12 (40.0 %)	11 (36.7 %)	11 (36.7 %)	6 (20.0 %)	
Gender					
Male, *n* (%)	9 (30.0 %)	8 (26.7 %)	13(43.3 %)	14 (46.7 %)	0.292 ¥ NS
Female, *n* (%)	21 (70.0 %)	22 (73.3 %)	17 (56.7 %)	16 (53.3 %)	
BMI (Kg/m²)					

Mean ±SD	34.73± 3.95^{A}	24.91± 3.07^{B}	34.01± 4.38^{A}	22.75± 1.85^{B}	0.001 † S
Range	29.75 –44.08	15.63- 29.38	28.37- 45.79	19.81- 26.53	
Different letters denote the significant differences at p< 0.05					

n: number of cases; **SD**: standard deviation; †: one way ANOVA; ¥: Chi-square test; **NS**: not significant at $P > 0.05$

Both groups of patients (gallstone patients with obesity and patients with obesity only) showed a significant increase (p< 0.01) compared to the control group. In contrast, a non-significant difference (p>0.05) was found between gallstone patients without obesity and the control group. Also, a non-significant difference (p>0.05) was found between gallstone patients with obesity and patients with obesity-only groups themselves.

Previous studies, in which body mass index and serum lipids were studied by multivariate analysis, did find that BMI serves as an indicator of body fat, and supplementary measurements, such as waist circumference, can further aid in diagnosing obesity [19].

A previous study showed that BMI has a causal effect on gallstone risk. [20]. It has been estimated that a BMI increase of more than 5 points raises the risk of gallstone formation by 1.63 times [21].

All patient groups showed a significant increase in TG levels (p< 0.01) compared to the control group, whereas a non-significant difference (p>0.05) was found between gallstone patients with obesity and patients with obesity-only groups themselves.

All patient groups showed a significant increase in cholesterol, VLDL, and LDL levels (p< 0.01) compared to the control group, whereas a non-significant difference (p>0.05) was found between the patient groups themselves.

However, the mean levels of HDL were significantly lower (p< 0.01) in all patient groups compared to the control group, whereas a non-significant difference (p>0.05) was found between the patient groups themselves.

In this study, serum lipids were measured at a time when gallstone symptoms were already present. The effect of obesity on various lipid components was observed in this study. The results of total lipids clearly show much higher levels in obese patients as compared to controls [22].

A study by (Bhatti et al. 2001) found that all the parameters except serum HDL level showed a significant increase in obese persons while HDL level was significantly decreased [23]. This is supported by the increase in percent body fat associated with an increase in apo-B levels [24]. This supports the results of the current study.

Obese people are at a higher risk of gallstones [25]. (Hongliang et al. 2024) showed a significant correlation between the risk of gallstone formation and higher TG [26].

(Table 4) Results of lipid profile (Triglycerides, Cholesterol, VLDL, LDL, and HDL) in patients and healthy controls.

Characteristic	Gallstone with obesity n=30	Gallstone without obesity n=30	Obesity only n=30	Control subjects n=30	P
Triglycerides (mg/dl)					
Mean± SD	150.9 ± 33.3^{A}	128.51± 29.71^{B}	159.47± 34.5^{A}	66.2± 5.65^{C}	< 0.001 † HS
Range	100.3- 240.02	100.3 – 196.90	100.4 – 273.6	53.0 – 89.0	
Cholesterol (mg/dl)					
Mean± SD	193.33 ± 20.67^{A}	180.27 ± 14.5^{B}	175.67± 12.3^{B}	138.5± 6.36^{C}	< 0.001

Range	162.4- 270.4	161.0 – 210.5	142.0 – 200.0	111.0– 162.0	† HS
Very-low-density lipoprotein (mg/dl)					
Mean± SD	42.46 ± 6.11^{A}	44.48 ± 9.99^{A}	36.1± 3.16^{A}	10.66± 1.06^{B}	< 0.001 † HS
Range	29.72- 78.26	25.18 – 114.66	27.08– 76.98	4.50–30.00	
Low-density lipoprotein (mg/dl)					
Mean± SD	97.83 ± 18.89^{A}	89.0 ± 16.8^{AB}	85.52± 12.34^{B}	65.23± 11.31^{C}	< 0.001 † HS
Range	64.22- 200.40	50.94 – 148.45	71.5– 108.66	40.0 –110.0	
High-density lipoprotein (mg/dl)					
Mean± SD	33.02 ± 40.6^{A}	35.59± 4.56^{A}	30.88± 5.16^{A}	46.4± 6.36^{B}	< 0.001 † HS
Range	24.50- 44.20	22.30 – 48.70	24.5 – 40.0	30.0 – 66.0	
Different letters denote the significant differences at p< 0.05					

n: number of cases; **SD**: standard deviation; †: one way ANOVA; ¥: Chi-square test; **HS**: Highly significant at *P* < 0.001

All groups of patients showed a significant increase in CRP levels (p< 0.01) compared to the control group. Also, a significant increase in gallstone patients with obesity versus gallstone patients without obesity and patients with obesity only, whereas a non-significant difference (p>0.05) was found between gallstone patients without obesity and patients with obesity only.

CRP is considered a main inflammatory marker [27]. The increase of adiposity might be an essential factor in the expression of CRP and chronic inflammation leading to obesity [28].

Elevated CRP levels are frequently used as indicators to assess the severity of inflammation in gallbladder diseases, as gallstones can induce inflammation in both the gallbladder and biliary tract. This inflammation stimulates the liver to produce CRP [29].

(Table 5) C-Reactive protein levels in patients and healthy controls.

Characteristic	Gallstone with obesity n=30	Gallstone without obesity n=30	Obesity only n=30	Control subjects n=30	P
C-Reactive protein mg/dl					
Mean ±SD	12.69± 4.53^{A}	7.82± 3.13^{B}	6.75± 3.08^{B}	3.33± 1.45^{C}	0.001 † S
Range	4.25 –22.69	5.19- 22.04	3.56- 15.11	1.76- 6.30	
Different letters denote the significant differences at p< 0.05					

n: number of cases; **SD**: standard deviation; †: one way ANOVA; S: significant at *P* < 0.05

All groups of patients showed a significant increase in SPX levels (p< 0.01) compared to the control group, whereas a non-significant difference (p>0.05) was found between the patient groups themselves.

SPX levels were decreased in both adults and children with obesity and diabetes [30]. SPX is anorexigenic adipokine that can modulate adipogenesis and glucose metabolism by suppressing food intake or increasing energy expenditure [31]. Therefore, SPX may serve as a new therapeutic candidate for treating obesity [32].

The role of SPX in lipid metabolism was demonstrated in a study by Cheng-Yuan Lin et al. (2018), which found that SPX administration in rats resulted in a significant reduction in serum total bile acids and the expression of cholesterol 7α-hydroxylase 1 (CYP7A1) in the liver. This suggests a potential role for SPX in bile acid synthesis and lipid metabolism [13].

As a result, SPX has a potential role in regulating these pathways and may offer a new avenue for the treatment or prevention of gallstones in the future.

(Table 6) Spexin level in patients and healthy controls.

Characteristic	Gallstone with obesity n=30	Gallstone without obesity n=30	Obesity only n=30	Control subjects n=30	P
Spexin level pg/ml					
Mean ±SD	85.5± 13.5^{A}	70.45 ± 12.1^{A}	101.6± 15.2^{A}	227.8± 15.6^{B}	< 0.001 † HS
Range	26.75 –173.5	11.75- 101.25	49.25- 206.75	62.0- 339.75	
Different letters denote the significant differences at p< 0.05					

n: number of cases; **SD**: standard deviation; †: one way ANOVA; **HS**: Highly significant at *P* < 0.001

Both groups of patients (gallstone patients with obesity and patients with obesity only) showed a significant increase in HMGCR (p< 0.01) compared to the control group. In contrast, a non-significant difference (p<0.05) was found between gallstone patients without obesity and the control group.

A study by Lau and colleagues (2018) found that HMGCR activity is elevated in the livers of obese individuals, leading to a higher production of cholesterol and fat [33].

A relation exists between HMGCR expression activity and gallstone formation [34]. Studies have shown that obese individuals have elevated cholesterol production and an increased risk of developing cholesterol gallstones. This is linked to enhanced HMGCR activity, which promotes cholesterol overproduction [35].

A previous study showed that this enzyme's gene expression level increases in cases of obesity and gallstones, but it is higher in obese patients [36].

(Table 7) Gene expression in patients and healthy controls.

Characteristic	Gallstone with obesity n=30	Gallstone without obesity n=30	Obesity only n=30	Control subjects n=30	P
Gene expression					
Mean ±SD	2.01 ± 0.49^{A}	1.20 ± 0.38^{B}	1.71± 0.47^{C}	1^{B}	< 0.001 † HS
Range	1.25 –3.61	0.76- 2.64	0.81- 3.48	0.63- 1.2	
Different letters denote the significant differences at p< 0.05					

n: number of cases; **SD**: standard deviation; †: one way ANOVA; **HS**: Highly significant at *P* < 0.001

About correlations, there is a significant negative correlation between SPX with BMI, and Cholesterol (r=-0.458, p=0.001),

(r=-0.334, p=0.031) respectively in gallstone patients with obesity.

A previous study found that serum SPX levels were negatively correlated with BMI [37].

Negative correlations were observed between serum SPX and total cholesterol (TC) which may also be associated with the anti-obesity function of SPX [13].

There is a significant negative correlation between SPX and Cholesterol, (r=-0.387, p=0.002) in patients with obesity only.

There is a significant negative correlation between HMGCR with SPX, BMI, TG, and CRP (r=-0.564, p=0.001), (r=0.419, p=0.001), (r=0.492, p=0.001), and (r=0.413, p=0.001) respectively in gallstone patients with obesity.

The current study identified a significant negative correlation between HMGCR and SPX. Although direct evidence for a correlation between HMGCR and SPX is currently lacking, their involvement in metabolic regulation hints at a potential indirect influence between them, particularly in lipid metabolism and energy balance. Further research examining their interaction, especially with metabolic disorders such as obesity, could offer important insights into their possible connection.

HMGCR has been associated with BMI regulation in several genome-wide associations (GWA) [38]. Furthermore, studies involving statins indicate that central HMGCR activity may play a role in maintaining BMI [39].

HMGCR promotes an increase in total cholesterol and triglyceride levels [40]. Belfiore et al. (2017) demonstrated that elevated HMGCR activity was associated with increased TG levels in individuals with regulating lipid metabolism disorders [41].

Benedetta et al. (2023) identified a link between HMGCR and CRP polymorphisms and serum metabolic and inflammatory parameters in healthy adolescents. Their findings suggest that these polymorphisms could serve as risk factors influencing the metabolic and inflammatory profiles in this age group [42].

There is a significant negative correlation between HMGCR with SPX, BMI, VLDL, HDL, and Cholesterol (r=-0.331, p=0.011), (r=0.620, p=0.001), (r=0.405, p=0.001), (r=-0.441, p=0.001), and (r=0.281, p=0.019) respectively in gallstone patients without obesity.

Burnett et al. (1997) examined how atorvastatin, an inhibitor of HMG-CoA reductase, affects VLDL metabolism in miniature pigs. They found that atorvastatin's inhibition of HMG-CoA reductase led to a reduction in VLDL apolipoprotein B (apo-B) levels, mainly by decreasing apoB secretion into the plasma [43]. These results suggest that HMG-CoA reductase may play a direct role in the synthesis and release of VLDL particles.

A study also found that HMGCR expression correlated negatively with serum HDL levels [44].

Since HMGCR is the rate-limiting enzyme in cholesterol biosynthesis, directly influences total cholesterol levels [34]. Elevated HMGCR activity leads to increased cholesterol synthesis, thereby raising total cholesterol concentrations. HMGCR can cause metabolic disorders and raise cholesterol levels [45].

4. CONCLUSION:

The current study showed an increase in lipid profile and a decrease in HDL levels in each group of patients which coincided with increased activated inflammation represented by CRP which is accompanied by a decrease in SPX levels and also shows a rise in HMGCR gene expression.

These factors may be critical in developing gallstones through specific mechanisms, especially in obese patients. Inhibiting these pathways by determining their mechanism of action may provide promising treatments for gallstone patients.

Comprehensive studies are needed to evaluate SPX levels and HMGCR gene expression in diagnosing Cholelithiasis.

AUTHORS CONTRIBUTION:

Rusul Ayar Kassim and Dr. Nawal Khinteel Jabbar participated in designing the study, analyzing the results, and drafting the report. The authors have given their approval to submit the final version.

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