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Study of risk factors and liver function markers in gallstone patients in Iraqi Kurdish Women

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ABSTRACT

According to reports, gallstones are solid clumps of various sizes that grow in the biliary tract, and gallbladder cholelithiasis has been linked to some risk factors. Some countries have a higher prevalence of gallbladder stones than others. The study included (81) female subjects aged 20 to 70 with symptomatic and asymptomatic gallstones and (80) without gallstones. The goals of the current study were to find the association of risk factors (age, diet, family history, body weight, marital status, occupation, smoking, physical inactivity, parity, and education levels) with gallstones and determine the liver function markers in gallstone patients. The SPSS program analyzed the data using the chi-square and unpaired t-tests; $p < 0.05$. was regarded as statistically significant. The highest ratio of gallstones at age (41-50) years representing 34.57% of the patients, and the lowest distribution was observed in the age groups between (20-30) and (51-60) years that represent (12.35%) of patients. The patients with unhealthy diets represent (83.95%) and (16.05 %) of healthy diets in enrolled patients. The gallstone patients with a family history of gallstone described (55.56%) and (44.44%) were without a family history. Overweight and obese represented (87.65%) of patients, and most of the cases were inactive groups, multiparous; illustrate, the difference between the cases and controls was statistically significant. Alanine transaminase (ALT), Aspartate aminotransferase (AST), bilirubin, fasting blood glucose (FBG), waist circumference, body mass index (BMI), systolic blood pressure (SBP), diastolic blood pressure (DBP), were significantly higher in cases than control. We concluded that women with high body weight, physically inactive, multiparous women, and less educated are more prone to develop Cholelithiasis and ALT, AST, BMI, waist circumference, SBP, and DBP associated with gallstone.

Keywords: Gallstone disease, cholelithiasis, risk factors, family history.

1. Introduction

Gallstones are solid clumps of various sizes that grow in the biliary tract and gallbladder. they are formed of proteins, mucin gel, calcium bilirubinate, and cholesterol monohydrate crystals. Some people get numerous little gallstones, whereas others develop only one or a few large ones[1]. It is the most frequent biliary system condition that requires hospitalization[2].

Gallstones are primarily asymptomatic. When there are symptoms, the condition is typically not life-threatening; it can lead to severe and potentially life-threatening complications, such as acute cholecystitis, acute cholangitis, and biliary pancreatitis.The clinical characteristics include upper right abdominal pain, together with more frequently occurring nausea, vomiting, and sensations of fullness after meals, which harm the patient's quality of life[3]. Sometimes a dull colicky ache that could radiate to the shoulder and lasted for more than 30 minutes[4].

Cholesterol, pigment, and mixed gallstones are the three different forms of gallstones based on their primary biochemical components[5]. The majority of gallstones, which are yellow or grey stones made of crystalline cholesterol monohydrate, are caused by cholesterol[6].

Gallstone disease affects 10–20% of the world's population[7]. Cholelithiasis has been linked to many risk factors, according to reports. Factors like aging, female sex, pregnancy, rapid weight loss, lipid disorders, genetic background, and lifestyle are all recognized as a risk factors for forming cholesterol gallstones caused by increased synthesis and secretion of cholesterol[8]. Type two diabetes[9], obesity[10], and cigarette smoking[11] have been regarded as risk factors.

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For early gallstone identification, ultrasonography is favored as a screening method in older individuals, female, have high lipid levels, a family history of gallstones, a high body mass index, and co-morbid conditions like diabetes or hypertension[12].

Gallstones are formed when bile contains excessive amounts of cholesterol and other enucleating agents, such as calcium salts of bilirubin; they are generally divided into three categories (based on the presence of cholesterol): cholesterol stones, mixed stones, and pigment stones[13, 14]. According to epidemiological studies, cholelithiasis is more prevalent in adult females than males[7]. The rate of gallstone formation in women is approximately two times more than the male[15]. Gender is a well-known risk factor for cholelithiasis may be due to the modification in the composition of bile and stasis in the gallbladder caused by the production of estrogen and progesterone[16].

The association between age and gallstones documented that above age 40, the occurrence of gallstones increases four times more than in younger people, and between 40 and 60, the high numbers were female[17]. Gallstones are primarily observed in patients over 65 and are higher in females[18]. Marriage is associated with an increased incidence of gallstones[19] multiparous, with 4-5 children more prone to cholelithiasis[17]. Pregnancy is linked to gallstone disease, and the chance rises with multiparity[16]. Multiparity was observed in most female patients (71%)[20].

An unhealthy diet also affects gallstone formation; for instance, consuming carbohydrates induces the incidence of pigment gallstones, whereas eating meat and fried foods with fat increases the chance of cholesterol gallstones[21]. Evidence also shows that eating fruits and vegetables lowers the risk of gallstones, while eating spicy foods and cooking with oil raises the risk[22]. Obese people are more likely to get gallstones with a higher body mass, and a higher chance of developing cholesterol gallstones is associated with excess body weight[23].

Occupation also affects gallstone formation, and homemakers may be related to gallstone formation[17]. A family history of gallstones also showed a significant association with gallstones[24]. Individuals with one relative with gallstones are at almost double the risk of developing gallstone complications, but the risk increases with more relatives with gallstones[25]. Regular physical activity reduces the incidence of chronic diseases, particularly cardiovascular diseases and gallstone formation[26]. Type 2 diabetes, cardiovascular disease, hypertension, and metabolic syndrome are highly related to the waist-to-hip ratio[27]. The advantage of the waist-to-hip ratio over other anthropometric markers for assessing obesity is supported by several underlying mechanisms[28]. However, it is still uncertain whether various obesity indicators for GSD, such as the waist-to-hip ratio, are predictable.

Liver damage may also result from a gallstone that develops a blockage in the bile duct and causes bile congestion. Transient ampullary blockage, which causes an abrupt increase in bile duct pressure and subsequent liver cell destruction, is the most likely reason for the early and temporary elevation in ALT[29].

In the Iraqi Kurdistan region, the study of gallstone disease and its associated risk factors have not been studied yet or little studied. Therefore, the present study aimed to identify the association of risk factors on cholelithiasis and study of liver function parameters in Iraqi Kurdish women with gallstones. A better knowledge of the risk variables may assist us in identifying patients with gallstone disease (GSD) and lowering the risk of GSD in some cases. As a result, we conducted case-control research in the Kurdish women population to determine risk factors for GSD.

2. Materials and Methods

2.1 Study design and setting

The study was a case-control study; data were collected from patients visiting Garmian Medical Centre, Kalar, Sulaymnia, Kurdistan, Iraq. All subjects were approached after the clinical diagnosis in the hospital, based on the clinical examination they had undergone and ultrasonography inside or outside the hospital, after obtaining agreement from each patient to participate in this study.

2.2 Study participants and sampling

The study included 161 female subjects, all at age (20-70) years. Eighty-one with symptomatic and asymptomatic gallstone disease and eighty healthy female groups were judged to be clinically free from gallstone disease, and they were chosen as a healthy group for comparison of biochemical factors associated with gallstone. Who attended the hospital from June 2021 to October 2022.

2.3 Data collection tool and technique

More information was collected from each patient by filling out a prepared study form. Interviewers conducted face-to-face interviews with each sample using a validated questionnaire form. The survey asked about risk factors such as age, family history, marital status, occupation, smoking, physical activity, parity, and education levels. Waist circumference, waist-to-hip ratio, Height, weight, and BMI were calculated with a standard formula. In addition, systolic and diastolic blood pressure were measured.

2.4 Study of variables

The dependent variable was Gallstone disease, and the independent study variables were included. The continuous variables were age, weight, height, BMI, blood pressure, waist circumference**,** and liver functions parameters. The categorially variables were consumption of a healthy diet, family history of Cholelithiasis, marital status, occupation, smoking, physical activity, parity, and education levels.

2.5 Inclusion criteria

Female sex aged 20 -70 years or older, willingness to participate in the study, and approval of GSD were the requirements for inclusion. For controls, the same characters mentioned above but without gallstone and family history of gallstone.

2.6 Exclusion criteria

Males were omitted entirely, and females less than 20 years, intestinal disorders, known autoimmune diseases, cancer,

pregnancy or lactation women, or unwilling to continue cooperating were excluded.

2.7 Ethical Consideration

The Ethical approval letter was obtained from the scientific and ethical committee of the Department of Biology, College of Education, and the University of Garmian. All patients provided informed consent to participate in this study; each participant was informed about the study's objectives. The study was carried out under the supervision of the ethical committee of the University of Garmian (Ethical approval No.5: 20/06/2021), which followed the declaration of Helsinki.

2.8 Biochemical analysis

5 ml of blood was drawn from each participant, then placed in a collecting tube with gel and clot activator but no anticoagulant. Accredited laboratories then performed measurements on the samples. The levels of (ALT, AST, ALP, FBG, Bilirubin and Albumin) were determined using an automatic biochemical analyzer (Cobas c 111) at the pharma lab in Kalar.

3. Statistical analysis

A Microsoft Excel spreadsheet was used to enter the data. The analysis used numbers and percentages (%) to arrange categorical variables. Categorical variables such as marital status, occupation, smoking, physical activity, parity, and education level were compared between case and control subjected to the Chi-square test using a graph pad prism. Results of liver functions ALT, AST, ALP, Bilirubin, Albumin, FBS, Total protein, Age, Waist circumference, Waist /Hip, Height, Weight, BMI, systolic, and diastolic blood pressure are displayed using Mean ± SEM, an unpaired t-test was used to compare the parameters between the two groups using IBM's Statistical Package for Social Sciences (SPSS) software, version 21.0 (IBM, Chicago, IL, USA).

4. Results

In this study, as in Table (1), A high ratio of gallstones was observed at age (41-50) years, which represented (34.57%) of the enrolled patients, the patients with unhealthy diets expressed (83.95%) of the enrolled population, and the lowest percentage was healthy diet subjects, about (16.05 %) of enrolled patients. The rate of gallstone patients with a family history of cholelithiasis was about (55.56%) and about (44.44%) were without a family history. The ratio of gallstone formation with the body weight patients with overweight and another degree of obesity represent a higher percentage (87.65%) of the enrolled population, and the lowest rate of the patient were normal weight subjects, about (12.35%) of enrolled patients.

Table 1: Some characteristics of gallstone formers.

Variables	Frequency(n)	(%)
Women's Age (Year)		
20–30	10	12.35
31–40	20	24.69
41-50	28	34.57
51-60	10	12.35
61-70	13	16.04
Mean	45.41	
Diets		
Healthy diets	13	16.05
Unhealthy diets	68	83.95
Family history of cholelithiasis		
With family history	45	55.56
With-out family history	36	44.44
BMI		
Normal weight	10	12.35
Overweight	28	34.57
Moderate obese	28	34.57
Severely obese	12	14.81
Very severely obese	3	3.70
Total	81	100%

BMI: body mass index

In Table (2), the result presented the highest ratio of gallstone formation in married patients (91.36%) of female patients, and the lowest percentage was detected in unmarried patients (8.64%) of the females with GSD patients. Still, there was a statically non-significant difference between the cases and controls. Regarding the formation of gallstones concerning occupation, the percentage of gallstone patients who were housewives is about (74.07%), and about (25.93%) of the patients were employees. In comparison, in control, there were (72.50%) homemakers and (27.50%) employees. So statically, no significant difference was observed.

No significant association was observed between gallstone formation and smoking in the enrolled female gallstone population; the high prevalence of gallstone formation was seen in the nonsmokers, representing about (96.30%) and (97.50%) of controls of females, whereas the smokers represented about (3.70%) and (2.50%) respectively nearly the same ratio was found in both groups. The results of the effect of physical activity on gallstone formation indicate a lower proportion of the physically active group (16.05%) of enrolled patients. At the same time, the highest ratio was a physically inactive group, representing (83.95%) of enrolled patients; there was a significant difference between the two groups (p-value 0.001). The ratio of multiparous was the highest (82.72%) of the female patients and the lowest in uniparous females, who made up (7.40%) of the female patients. The difference was significant between the two groups (p-value 0.0046).

Based on education level, the current study demonstrated that gallstone production is more common in illiterate patients when compared with other education levels; the highest ratio was (55.56%) an illiterate female patient. The lowest was in secondary school (4.94%) of the female patients; the difference was significant between the two groups (p-value 0.0013).

Table 2: Comparison of some risk factors between cases and controls.

Variables	Cases, (N=81), ***n*** **(%)**	Controls, (N=80), ***n*** (%)	P value
Marital Status			
Married	74 (91.36)	70 (87.50)	0.4258

Single	7 (8.64)	10 (12.50)	
Occupation			
Housewife	60 (74.07)	58(72.50)	0.8214
Employee	21 (25.93)	22(27.50)	
Smoking			
Smokers	3 (3.70)	2 (2.50)	0.6598
Nonsmokers	78 (96.30)	78 (97.50)	
physical activity			
Active Group	13 (16.05)	50 (62.5)	0.001*
Inactive group	68 (83.95)	30 (37.5)	
Parity			
Nulliparous	8 (9.88)	21(26.25)	0.0046*
Uniparous	6(7.40)	11(13.75)	
Multiparous	67(82.72)	48(60.00)	
Education Level			
Illiterate	45 (55.55)	34 (42.50)	0.0013*
primary school	19 (23.46)	8 (10.00)	
Secondary school	4 (4.94)	7 (8.75)	
Institute	6 (7.44)	16 (20.00)	
University	7 (8.61)	15 (18.75)	

N: subject number, P value calculated using chi-square test, *significant

The results of the current data in Table (3) showed a significant difference in serum ALT and AST levels and a non-significant difference in the level of ALP in gallstone patients compared to controls. On the other hand, the levels of the liver non-enzymatic parameters show a significant elevation (P< 0.05) in the bilirubin and albumin level in gallstone patients compared to the control group. At the same time, glucose levels showed a significant elevation (P< 0.05) in gallstone patients compared with a healthy control group. At the same time, the level of serum total protein in patients with gallstones was non-significantly changed compared to the control **(Fig1 and Fig2).**

Table 3: Comparison of liver functions between controls and gallstone patients (mean ± S.E).

parameters	Control mean ± S.E.	Patients mean ± S.E.	*p* Value
ALT (U/l)	18.9 ± 2.08	29.71 ± 2.61	0.002*
AST (U/l)	19.25 ± 1.03	28.94 ± 4.06	0.046*
ALP (U/l)	72.22 ± 1.03	77.65 ± 4.77	0.447
Bilirubin (mg/dl)	0.47 ± 0.03	0.68 ± 0.05	0.005*
Albumin (gm/dl)	4.80 ± 0.11	4.23 ± 0.07	0.000*
FBG (mg/dl)	96.56 ± 1.99	124.38 ± 4.29	0.000*
Total protein (g/dL)	6.51 ± 0.18	7.04 ± 0.20	0.059

ALT: Alanine transaminase, AST: Aspartate aminotransferase, ALP: Aspartate aminotransferase, FBG: fasting blood glucose, P value calculated using unpaired t-test, *significant

Table 4. showed that patients with GSD had significantly higher waist circumference (100.66 cm vs. 93.67cm), BMI (30.44 vs. 27.91), systolic bp (127.53 mmHg vs. 116.71 mmHg), and diastolic bp (83.46 mmHg vs. 78.71 mmHg) than those without GSD, (all $p < 0.05$).

Table 4: Mean differences in age, Waist, Hight, BMI and blood pressure variables in gallstone patients and controls (mean ± S.E).

variables	Control mean ± S. E	Patients mean ± S. E	p Value
Age	43.13 ± 10.49	45.41 ± 13.02	0.225
Waist circumference, cm	93.67 ±1.74	100.66 ±1.89	0.008*
Waist /Hip	0.91 ± 0.08	0.92 ± 0.05	0.401
Hight, m	1.61 ± 0.008	1.59 ± 0.009	0.154
Weight, kg	73.27 ±1.77	77.66 ±1.70	0.079
(BMI) kg/m^2	27.91 ± 0.84	30.44 ± 0.56	0.011*
Systolic bp mmHg	116.71 ± 1.93	127.53 ±1.94	0.000*
Diastolic bp mmHg	78.71 ± 1.439	83.4 6±1.31	0.031*

S.E: Standared error, Cm: centimeter, m: meter, kg: kelogram, BMI: Body mass index,
* A P-value of <0.05 is considered significant,

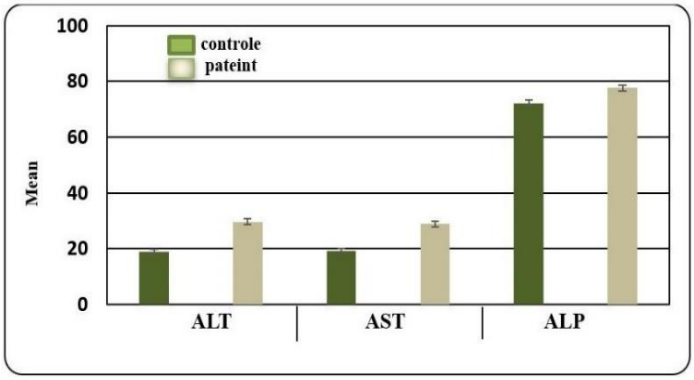

Figure 1: Liver function enzymes in gallstone disease.

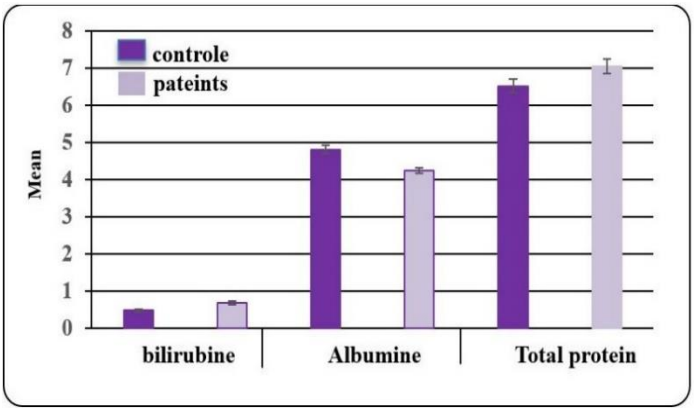

Figure 2: Non-enzymatic liver function parameters in gallstone disease.

5. Discussion

The present study included females only because gallstones mainly occur in women. A similar study concluded that the high incidence of gallstones in women is more common than in men[7].

Female gender was considered a significant risk factor for gallstones; other studies confirmed the same results[24]. In previous studies, the condition was found to be more common in women, which was also reported. It could be related to the estrogen hormone increasing the cholesterol supersaturation in the bile, which leads to the development of gallstones. The mean age of the patients was (45.41 ±13.01) years. The nearly same result was found in an investigation by Hayat et al[30] with mean ages of 40.90 ± 12.65 years. However, the study found that the patients' mean age was (48.19.7) years[31].

The age of the patients with gallstones ranges from 20 to 70 years. The 31-40 and 41-50 have a higher frequency. Chou showed a similar frequency[28]. As people age, Other risk factors could assemble and result in gallstone development. Biliary cholesterol secretion increases. The production of bile acid reduces. Cholesterol becomes more saturated. These adverse side effects result from a reduction in the role of cholesterol 7a-hydroxylase (CYP7A1), the rate-limiting enzyme for bile acid synthesis[32]. Our study found a remarkable association between diet habits and gallstones because 83.95% of patients consume an unhealthy diet, especially a diet rich in fat. Many studies revealed this relationship between fatty diet and gallstones. Dietary cholesterol increases biliary cholesterol saturation and raises gallstone formation.

An unhealthy diet also affects gallstone formation. For instance, consuming carbohydrates increases the frequency of pigment gallstones, but eating fried and meat-based fat increases the likelihood of developing cholesterol gallstones[21].

Regarding dietary habits, some evidence indicates that eating vegetables and fruits has a negative relationship with gallstone disease, whereas eating spicy foods and heating oil raises the risk[22]. This population consumes a diet containing more fat so that dietary habits may participate in gallstone formation.

Fifty-five out of 81 patients (55.56%) had a family history of gallstone disease, similar results were found by others[17, 24, 33] that confirmed the occurrence of cholelithiasis associated with a family history of gallstone disease. Disagreement with several previous studies reported that biliary stones are associated without a family history of gallstones in various populations[4,34,35]. Some studies suggested that genetics has a significant role in the development of gallstones, where increased frequency of gallstones in the first degree of relatives is a risk factor[25]. Shared lifestyle and metabolic factors may also contribute to the inherited susceptibility to gallstone disease.

We did not find a significant difference in the case of marital status; this agrees with the result by Gu[29]; in contrast, the study concluded that the risk was three times higher for single people than married people[36]. Female sex hormones, specifically estrogen, activate hepatic lipoprotein receptors, causing dietary cholesterol to be absorbed and biliary cholesterol to be secreted. Reduced gall bladder contraction is brought on by progesterone.

The formation of gallstones concerning the occupation in GSD patients was shown; the percentage was homemakers in cases and controls, which are (74.07%) and (72.50) respectively. So statically non-significant difference was found between the patient and control. Similar results have documented that occupation had related to gallstone formation[35]. Most women in our region may be home workers; only some are employed, so they stay home most of the time with less activity.

In this study small ratio of participants were smoking 3.70 % of patients and 2.47 % of controls; there is statically non-significant. A similar study demonstrated that smoking was not a significant risk factor in gallstones[36]. In contrast to this result, some substantial correlation between smoking, a history of gallstone disease, and the formation of gallstones has been shown in epidemiologic research in the past[37]. Nicotine has been linked to more cholesterol crystallizing in the bile and less mucin being made and released by the gallbladder. Because nicotine lowers the production of prostaglandin, also, another study showed that smoking disrupted gallbladder contractility[38]. Our result was acceptable because females in the Kurdish population did not have a smoking habit in both the gallstone patients and control groups.

Our study documented that physical inactivity has an important role in gallstone formation, similar to most previous studies, which demonstrated the protective effects of sports on gallstone formation[39, 40]. So Physical activity improves intestinal motility, increases bile acid excretion, and prevents gallstone formation.

The results of this study suggested that multiparous women with two or more children may be more prone to developing stones than nulliparous or uniparous women. This result was in line with other earlier studies that indicated a good correlation between multiparity and GSD[41]. Estrogen increases the secretion of biliary cholesterol, which causes the bile to become too saturated with cholesterol and lithogenic[42]. And sluggish gallbladder motility, both of which promote gallstone formation[43]. Moreover, our observations showed that pregnancy or parity is a significant risk factor for gallstones, which agrees with other investigations[36]. On the other hand, previous work declines to suggest such a relationship between parity and gallstones. It may be due to hormonal changes during recurrent pregnancy that aid gallstone production.

According to education level, the result of the current study demonstrated that gallstone production is more common in an illiterate patient when compared with another education level of GSD patients; this observation agreed with the results of several previous studies[35, 40]. A different study found that women with poor education levels were more likely to have gallstones[24]. So, the education level protects humans from gallstone formation through the arrangement of lifestyle feeding habits.

Patients with elevated ALT levels may have abnormal liver function, which reflects the health status of the liver. The results of the present study indicated a positive statistically significant association between ALT, AST, serum total bilirubin levels and GSD patients. The result was in line with a study that revealed the relationship between GSD patients and the level of liver function enzymes[44]. While the consequence showed a non-significant association between gallstone (GS) formation and

serum ALP level, result agreement with the result of studies showed that serum levels of ALP tended to be elevated in individuals with GSD, but the association did not establish a statistically significant between GS formation and the high levels of this enzyme[33]. The serum albumin levels were significantly decreased in gallstone patients, and Similar results found an association that matched the one discovered in the current study[19]. Regarding blood glucose, the result of the present study showed a statistically significant elevation of glucose levels in gallstone patients. The results were in line with the results of most previous studies that showed a high prevalence of GSD among people with diabetes[15, 45]. Factors were suggested to be associated with increased gallstone prevalence in diabetic patients included supersaturating of bile, changes in the cholesterol nucleation and decreased motility of the gallbladder.

We noted that the body mass index (BMI) was significantly higher in cases than controls. Many studies indicate that the relative risk for GSD is considerably higher in people who are the most obese[46]. Obesity is associated with cholesterol supersaturation in the bile and decreased gallbladder contractility with meals, which may lead to lethogenesis[47].

Gallstone development may be affected by the body's fat distribution compared to total body fat. In both female and male patients, abdominal obesity is associated with several metabolic diseases, including the formation of gallstones. In our study, waist circumference and BMI were significantly higher in GSD patients; these results align with the study by Chou et al.[28].

We found no significant difference in the waist-to-hip ratio between cases and controls. Still, Balakrishnan et al.[48], have reported an increase in the waist-to-hip ratio in patients compared to the control. It is because we had studied only females, while studies on both genders may lead to a difference between the reports. We discovered a substantial rise in blood pressure in cases compared to controls. A study found that cholelithiasis patients with gallstone disease had higher SBP and DBP than controls[48]. The inconsistent result was found in the previous research by Khalaf et al.[24].

Limitation and Recommendations

The results of our study merit further evaluation with a larger sample size, and the study included only females. Health education is immediately required to make modest lifestyle modifications and reduce the prevalence of cholelithiasis in females. We recommend another gallstone study that includes a genetic analysis contributing to gallstone formation.

Conclusions

Risk factors for gallstones are multifactorial. We conclude that inactive individuals, multiparous women, and low education level individuals are more likely to develop gallstone formation in women. In addition, a high body mass index and an increased waist circumference are risk factors. The liver functions, and blood pressure in females with cholelithiasis was associated with gallstones. Health education is immediately required to make modest lifestyle modifications and reduce the prevalence of cholelithiasis in females.

Conflict of interests

None

Author contribution

Data and sample collection, laboratory works, data analysis and draft writing were done by Seerwan Assi Raheem; Ismail Salih Kakey did supervision and review and editing.

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