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Association Between Apolipoprotein E Gene Polymorphism and Gallstone Disease in Sulaymaniyah Governorate Women

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Abstract

Gallstone disease (GSD) is a worldwide problem; the frequency of GSD varies greatly amongst different ethnic groups, possibly due to environmental and genetic factors. One circulating lipoprotein involved in lipid metabolism is apolipoprotein E (APOE). The APOE gene is polymorphic, has three distinct alleles, APOE2, APOE3, and APOE4. The an APOE polymorphism is linked to a variety of diseases, including Alzheimer's and type-two diabetes, so this study aimed to assess the relationship among various APOE genotypes and alleles with gallstone cases in Iraqi Kurdish women. The present case-control study involved 81 cases with laparoscopic cholecystectomy for gallstone disease and 80 healthy women without GSD. Genomic DNA samples were taken from blood to find the genotype of APOE using allele-specific sequence-primers and a method based on polymerase chain reaction (PCR). lipid profiles were examined using an automated biochemical analyzer. SPSS and GraphPad Prism 9.1.1 software were used to conduct the statistical analysis. No significant relationships were found between healthy people and GSD patients in genotype and allele frequencies. However, the genotype for E3/E4 and the APOE4 allele in GSD patients were higher than the controls (9.88% vs. 5.0% and 4.94% vs. 2.5%, respectively; P <0.05) but statistically nonsignificant. serum levels of cholesterol, low-density lipoproteins, and very low-density lipoproteins were higher, and the levels of serum high-density lipoprotein was lower than controls. These findings revealed no connection between the examined polymorphisms and gallstone cases. Additionally, it was demonstrated that gallstone disease and lipid parameters had a favorable relationship.
Keywords: Apolipoprotein E, APOE, Gallstone disease, Genotype, Polymorphisms.

الارتباط بين تعدد الأشكال لجين صميم البروتين الشحمي E (Apolipoprotein E), ومرض حصى المرارة عند النساء في محافظة السليمانية

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الخلاصه

مرض حصى المرارة هو مشكلة عالمية ، بحيث يختلف تواتر هذا المرض اختلافًا كبيرًا بين المجموعات العرقية المختلفة ، والتي قد تكون بسبب العوامل البيئية والوراثية. أن أحد البروتينات الدهنية والتي تدخل في عملية التمثيل الغذائي للدهون هو (**صميم البروتين الشحمي E,** Apolipoprotein E). إن جين (APO E) متعدد الأشكال ويحتوي على ثلاثة أليلات مختلفة وهي ، ApoE2 ، و ApoE3 ، و ApoE4. وأن جين (APO E) متعدد الاشكال يرتبط بمجموعة متنوعة من الأمراض ، بما في ذلك مرض الزهايمر والسكري من النوع الثاني ، لذلك تهدف هذه الدراسة إلى تقييم العلاقة بين الأنماط الجينية والأليلات APOE المختلفة مع حالات حصوات المرارة لدى النساء الكرديات العراقيات. تم تحديد التكرار لمتغير (Apo E) في ٨١ حالة ممن خضعوا لعملية استئصال المرارة بالناظور لمرض حصات المرارة و ٨٠ من الإناث الأصحاء. تم أخذ عينات الحمض النووي الجينومي من الدم للعثورعلى النمط الجيني ل APOE برايمرات خاصة وبتسلسل خاص بالأليل وطريقة تعتمد على تفاعل البوليميراز المتسلسل(PCR) ، وتم تحليل ملفات الدهون في مصل الدم باستخدام جهاز محلل الكيمياء الحيوي. وتم استخدام برنامج SPSS و GraphPad Prism 9.1.1لإجراء التحليل الإحصائي .ومع ذلك ، كان النمط الجيني E3/E4 وأليل E4 للمصابين بمرض حصى المرارة أعلى

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من مجموعة ومجموعه السيطرة (٩,٨٨٪ مقابل ٥,٠٪ و ٤,٩٤٪ مقابل ٢,٥٪ على التوالي). وبالرغم من ذلك، كانت الفروق الوراثية وتواتر الأليلات للمجموعات المدروسة غير معنوية إحصائيا. وكانت مستويات الكوليسترول الضار وكوليسترول الدم كانت اعلى بشكل ملحوظ في مرضى المصابين بحصى المرارة عن المجموعة المرجعية، وكان مستوى الكوليسترول الحميد في الدم منخفضًا بشكل ملحوظ في المرضى عن المجموعة المرجعية. كشفت هذه النتائج عن عدم وجود علاقة بين تعدد الأشكال التي تم فحصها وحالات مرض حصى المرارة. بالإضافة إلى ذلك ، تم إثبات أن هناك ارتباط وثيق بين مرض حصى المرارة ومؤشرات الدهون.

الكلمات المفتاحية: صميم البروتين الشحمي E ، APOE ، مرض حصي المرارة ، الأنماط الجينية ، تعدد الأشكال.

Introduction

(GSD) is a digestive tract disorders that affects people all over the world. It is highly frequent in Western nations, with a 10-20% frequency among adults [(1)]. Multiple genetic and environmental variables combine to cause GSD, which has a multifactorial etiology [(2)]. Previous studies have connected gallstones to age, gender, obesity, type2 diabetes, and insulin resistance [(3-5)]. Females showed the strong association between gender and gallstone disease, particularly during reproductive years [(6)]. Gallstones are solid aggregates of varied sizes composed of proteins, crystals of cholesterol monohydrate, mucin gel, and calcium bilirubinate that produced in the biliary tract and gallbladder, generally gallstone divided into three categories (based on the presence of cholesterol): cholesterol stones, mixed stones, and pigment stones [(7, 8)]. The three important mechanisms that cause gallstone formation: are gallbladder motility impairment, increased hepatic cholesterol release (bile supersaturation within the cholesterol in the gallbladder) and fast crystallization of solid cholesterol [(6)]. Gallbladder stones have recently become more common in the Iraqi population[(9)]. And Iraqi Kurdish women in Kurdistan region[(10)].

Several studies have concluded that genetics is a main cause in the development of cholelithiasis. Apolipoprotein E (APOE) is one candidate genes that can be studied in some diseases; for instance, apo e gene polymorphism association and cholelithiasis has been studied earlier, the study documented that the APOE gene which include E4 allele is related with a higher occurrence of gallstone production in gallbladder, mainly in older individuals [(11)].

Apolipoprotein E is a lipoprotein that circulates in the blood and participate in lipid metabolism [(12)]. Varied populations have different frequencies for these alleles [(13)]. Age, race, and geographic location all have an impact on the frequency distribution of APO E alleles. The most prevalent wild-type gene is E3, whereas mutant genes E2 and E4 are less prevalent [(14)].

APOE, consists of only 299 amino acids, synthesized by hepatocytes, astrocytes, macrophages, and stem cells[(15)]. It's found in a variety of lipoproteins, such as VLDL, HDL, and chylomicrons [(16)]. chromosome 19 which contain Exon 4 has two non-synonymous single-nucleotide polymorphisms (SNPs) that cause three primary allelic variants APOE2, APOE3, and APOE4, built up the polymorphic APOE gene [(17-19)]. By substituting cysteine for the amino acid arginine158, APOE2 differs from APOE3. Cysteine 112 is changed to arginine in APOE4, which distinguishes it from APOE3, and depending on APOE gene variants E2/E3/E4, human populations can be divided into six different genotypes (E3/E3, E3/E4, E3/E2, E4/E4, and E2/E2)[(20)]. Numerous studies demonstrated a relationship between the APOE and Alzheimer's disease [(15)], type-2 diabetes, dementia[(21, 22)] and COVID-19 disease [(23)].

Nonetheless, Gallstones and a change in serum lipids have been associated [(24)]. Since cholesterol makes up the majority of gallstones, studies on the etiology of GSD has long focused on the involvement of cholesterol metabolism in the process of gallstone production [(25)].A metanalysis recommended more studies on apo e polymorphism in relation to gallstones [(2)].

The high total serum cholesterol, triglycerides, low-density lipoproteins (LDL), and low amounts of high-density lipoprotein (HDL) are the major characteristics of hyperlipidemia. There is debate regarding whether hyperlipidemias and gallstones are related [(26)].

In this region there is no study or little study about the APOE gen polymorphism and gallstone even in female disease, Additionally, this research may aid in the completion of the worldwide map of APOE variations in female patients with gallstones.

In Sulaymaniyah Governorate, Iraqi Kurdistan Region the genetic polymorphism of apo e had been examined by Al-Jaf [(13)]. Since no APOE polymorphism among Iraqi Kurdish women with GSD has been studied, more study on the APOE gene polymorphism and gallstones in women with GSD is required. The study's objectives were to determine whether APOE alleles were associated with gallstones in Iraqi Kurdish women with GSD and to provide details for future study on the association of APOE distribution with various health problems. The study also demonstrates the lipid abnormalities in women with GSD.

Materials and Methods

Study population

Eighty-one female patients were enrolled with symptomatic and asymptomatic gallstone disease between the ages of 20 and 70, all of whom were under cholecystectomy at GMC (Garmyan Medical City, Kalar, Sulaymaniyah governorate, Iraqi Kurdistan region). The study also included eighty healthy females without gallstones; they confirmed their diagnosis by ultrasonography, so they were chosen as a healthy comparative group.

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The study was conducted between June 2021 and June 2022. Following a 12-hour overnight fast, Venus blood samples were drawn and preserved in two tubes, one with anticoagulant for DNA treatment and the second without anticoagulant for lipid profile testing. The Roche COBASS C111 Diagnostic Kit (USA) was used to conduct routine lipid profile testing with its fully automated chemical analyzer. A complete questionnaire was created to obtain all of the essential data from the patient's records, such as age, BMI, and clinical information for comorbidities such as hyperthyroidism, diabetes, and hypothyroidism.

DNA extraction

DNA of all participants were extracted by using (Addbio, South Korea) kit. According to manufacturer's instruction whole genome DNA was isolated from blood, by adding 20 µl of proteinase k to each tube containing 200 µl of blood and permitted to incubate at 56 ◦C for 10 min, for elution of the genomic DNA 200 µl of elution buffer was used then stored at- 30°C until they were used.

APOE genotyping

In this investigation, the genotyping of Apo E was done using sequence-specific primers. Four primers that are allele-specific (Macrogen Co., Seoul, KR) which involve Primer number one for APOE-112cys is Forward and nucleotide sequences are (CGG ACA TGG AGG ACG TGT); Primer number two for APOE-158arg is revers and nucleotide sequences (CTG GTA CAC TGC CAG GCG); Primer number three for APOE-158cys revers nucleotide sequence (CTG GTA CAC TGC CAG GCA); Primer number four for APOE-112arg which forward nucleotide sequence (CGG ACA TGG AGG ACG TGC). Those primers were previously produced and verified [27]. Apo E genotyping in general Iraqi Kurdish has recently studied [13]. Each APOE haplotype was determined using two allele-specific sequence-specific primers: p1 and p3 for amplifying E2, p1 and p2 for amplifying E3, and p2 and p4 for amplifying E4. Genotyping of each DNA sample required three separate PCR reactions. Additionally, Each reaction includes a set of control primers that amplifies a 406 bp area on chromosome 9 internal control amplicon[28].

Polymerase chain reaction and gel electrophoresis

To amplify genomic DNA, thermocyclers (conventional PCR) reactions performed (Nexus, Eppendorf AG, Germany). The PCR mixture was processed by using the following component: five microlite of master mix (Addbio, South Korea), five microlitre of sample DNA, 0.8 µl (10 pmol) allele-specific primers (0.4 µl of each forward and reverse primer), and 0.1 (10 pmol) µl of each control primer, the overall reaction volume equal 11 µl , In place of the DNA sample, 5 l of nuclease-free water was utilized as the negative control in each procedure.. touch-down condition was used to prevent non-specific binding. The program of PCR condition includes the following steps. Initial denaturation for 5 min at 95°C followed by 5 cycles of denaturation at 96 ◦C for 20 s, annealing at 70 ◦C for 45 s, and extension at 72 ◦C for 25 s; then 21 cycles at 96 ◦C for 25 s, 65 ◦C for 50 s, and 72 ◦C for 30 s; and finally 6 cycles at 96 ◦C for 30 s, 55 ◦C for 60 s, and the last extension step was at 70 ◦C for 2 min[13]. Ten microliters of the PCR products were run on an agarose gel electrophoresis using 1.5% Tris-borate-EDTA/ethidium bromide gel (Simply, South Korea) to separate the PCR amplification products, The Imaging System Syngeneic (Synoptics Ltd., Surrey, United Kingdom) was used to take the gel photos under UV-light.

Statistical analysis

For the analysis of data, the two software, SPSS 28 (IBM, USA) and GraphPad Prism 9 (GraphPad Software, Inc., USA) were used. Each genotype and allele's frequency were calculated using the Fisher exact test. Two groups were compared using an independent t-test, and more than two groups were compared using a one-way ANOVA. Using a Tukey test as a post-hoc analysis for multiple comparisons, a p-value of 0.05 or less was used to determine a statistical significance.

Results and Discussion

This study revealed variations in gallstone disease distribution concerning some comorbidities. The results showed the highest prevalence of gallstones without comorbidities were 51 (62.96%), and those with comorbidities included hypertension 13 (16.05%), hypertensive with diabetes 3 (3.70%) and Hypothyroidism 7(8.64%). The demographic data includes age groups divided into ≤40 years 30 (37.4), >40 years 51(62.96), BMI divided into <25 represent (12.35%) and >25 years (87.65%) (Table 1).

Each DNA sample required three separate PCR reactions to determine its genotype. Allele existence was determined by the appearance of a 173-bp (E2, E3, and E4) band in PCR reactions. To ensure proper amplification by the control primers, each PCR reaction includes a 406 bp band (**Figure 1**). It is necessary to repeat unsuccessful reactions if neither the control amplicon (406 bp) nor the allele-specific amplicon (173 bp) were found.

Table 1. Comorbidities and demographic information of the patients with gallstones

Comorbidity	NO	%	Demographic data		
NON	51	62.96	Age groups.	NO	(%)

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HTN	13	16.05	≤40 years	30	37.04
DM	7	8.64	>40 years	51	62.96
HTN with DM	3	3.70	Total	81	100
Hypothyroidism	7	8.64	BMI (kg/m2)		
Total	81	100.00	≤25	10	12.35
			>25	71	87.65
			Total	81	100

Abbreviations: NON; without disease, DM; diabetes mellitus, HTN; hypertension, NO; number of participants

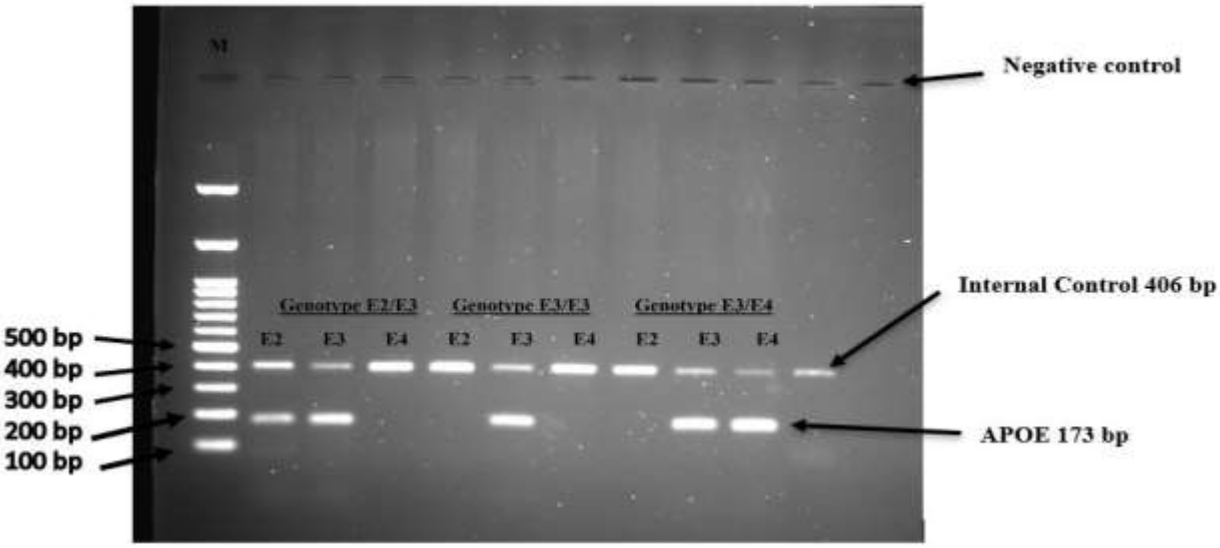

Figure 1.Agarose gel electrophoresis result of PCR: All three E2/3, E3/3, and E3/4.genotypes identified in this study: Primer forward 1 and Primer reverse 3 are present in E2. Primer forward 1 and Primer reverse 2 are present in E3. Primer reverse 2 and Primer reverse 4 are present in E4. Each primer pair will only create an APOE-specific 173-bp product if the matching allele is present. Additionally, each reaction contains a set of control primers that amplifies a 406 bp internal control amplicon; negative controls without DNA samples were used to improve the purity of the process. M=100 bp marker

The three alleles E2, E3, and E4, were observed in both patient and healthy groups, but, neither the healthy nor the GSD groups had any E2/E2, E2/E4, or E4/E4 genotypes. According to the data analysis there were no statistically significant differences between the six genotype frequencies of control people and GSD patients, even though there was a relative difference in the E3/E4 genotype (P = 0.3672) between the two groups. In contrast, there was no significant difference in the Apo E4 allele (P = 0.3781) in the frequency of alleles between GSD patients and control individuals. By comparing the two groups, there was no statistically significant difference between the Apo E2 and Apo E3 alleles, Table 2. lists the frequency findings from both healthy controls and GSD patients.

The comparison of lipid profiles between GSD patients and healthy control groups demonstrated a significant increase in total cholesterol, LDL, and VLDL levels, as well as a decrease in serum HDL levels. Furthermore, no significant change was observed in the levels of serum triglyceride between the two groups (Table 3). Comparisons between patients with and without comorbidities in terms of genotype and allele frequencies were also non-significant (Table 4), the serum lipid levels were estimated regarding to apo genotype. the result showed, no statistically significant differences were observed in the serum lipid value among the three deference genotype groups of gallstones (Table 5).

Table 2. Genotype and allele frequency of APO E gene polymorphism in cases and control subjects

Genotypes	Patients *N* = 81	%	Control N = 80	%	p-value	OR	95% CI
E2/E2	0	0.0	0	0.0	NC	-	-
E2/E3	7	8.64	6	7.5	0.7774	1.2374	0.4304 to 4.005

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E2/E4	0	0. 0	0	0. 0	NC	-	-
E3/E3	66	81.48	70	87.5	0.8160	0.93122	0.5858 to 1.4774
E3/E4	8	9.88	4	5	0.3672	2.1212	0.67425 to 6.5502
E4/E4	0	0.0	0	0.0	NC	-	-
Alleles	**N = 162**	**%**	**N = 160**	**%**	**p-value**	**OR**	**95% CI**
E2	7	4.32	6	3.75	0.7850	1.1905	0.43376 to 3.6756
E3	147	90.74	150	93.75	0.8723	0.9679	0.70911 to 1.32
E4	8	4.94	4	2.50	0.3781	2.0408	0.62640 to 6.1968

Abbreviations: N; Sample number, OR; Odds ratio, CI: Confidence interval.

Table 3. Serum lipid panel in gallstone and control subjects

Serum lipid profile	Gallstone mean ± S. E	Without Gallstone mean ± S. E	P value
Cholesterol (mg/dl)	192.04 ± 5.43*	170.61 ± 3.55	0.001
Triglyceride (mg/dl)	136.16 ± 5.93	126.53 ± 6.90	0.071
high density lipoprotein (mg/dL)	39.36 ± 0.96*	44.48 ± 2.04	0.028
low density lipoprotein (mg/dL)	142.13 ± 5.82*	96.46 ± 1.62	0.00
very low-density lipoproteins (mg/dL)	38.97 ± 1.44*	34.5 1± 0.80	0.015

Abbreviations: *Significant, S. E; standard error.

Table 4. Genotypes and allele distribution of APO E in GSD patients based to comorbidities

Genotypes	With comorbidities N = 30	No comorbidities N= 51	p-value	OR	95% Confidence Interval
E2/E2	0	0	NC	-	-
E2/E3	4	3	0.4170	2.333	0.57910 to 9.7561
E2/E4	0	0	NC	-	-
E3/E3	24	42	>0.9999	0.971	0.5008 to 1.5873
E3/E4	2	6	0.7046	0.583	0.11339 to 2.6320
E4/E4	0	0	NC	-	-
Alleles	**N = 60**	**N =102**	**p-value**	**OR**	**95% CI**
E2	4	3	0.4252	2.345	0.6075 to 9.5292
E3	54	95	0.9067	0.966	0.61481 to 1.5489
E4	2	4	>0.9999	0.789	0.16323 to 3.8813

Abbreviations: N; Sample number, NC; not calculated, OR; Odds ratio, CI: Confidence interval.

Table 5. lipid panels in GSD individuals with altered APO E allele distribution

Biochemical Parameter	E2/E3 mean ± S. E	E3/E3 mean ± S. E	E4/E3 mean ± S. E	P-value
Cholesterol (mg/dl)	192.83 ± 17.44	192.06 ± 4.83	191.12 ± 8.31	0.996
Triglyceride (mg/dl)	139.22 ±15.20	136.16 ± 4.08	133.50 ±13.28	0.949
High density lipoprotein (mg/dL)	43.19 ± 3.54	39.81 ± 0.97	38.90 ± 2.82	0.407
Low density lipoprotein (mg/dL)	155.08 ±23.13	144.50 ±7.45	141.48 ±15.42	0.889
very low-density lipoproteins (mg/dL)	39.54 ± 3.66	38.78 ± 1.22	39.86 ± 3.42	0.946

Abbreviations: S. E; standard error.

In the present study, APO E polymorphisms and lipid profiles were estimated in GSD patients and healthy subjects from Iraqi Kurdish women population. Patients and controls were Iraqi Kurdish, natives of the northern of Sulaymaniyah, who were matched for both age and all were women.

We have not observed any homozygous E2/2 or E4/4 or heterozygous E2/4 genotypes in the GSD patients, as it was hypothesized previously [13], The low incidence of (E2) and (E4) alleles in the local population may be due to low frequency of both alleles.

Although the APOE2/3 and E3/4 genotypes were more common in the GSD group than in the control group and the APOE3/3 genotype was less frequent, these differences were not statistically significant,

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and the outcomes were comparable. Numerous studies are in line with our results [29-31]. in contrast to this, a different study showed a link between gallstone disease and APOE [32].

The APOE allele frequencies were mainly steady for APOE2 and reduced for APOE3 in the patient group, while there were no appreciable differences. These findings therefore suggest that APOE2 has a neutral role, but APOE3 may play a protective one.

Though statistically non-significant, the APOE4 allele was twice as common in the GSD group as compared with control group. These findings imply that the APOE4 allele may participate in gallstone formation. Inconsistent association between (APOE-E4) and GSD were found in previous investigation [2]. However previous study have confirmed that the APOE4 allele directly contributes to cholelithiasis [33]. While the (E4) allele's frequency increased by the same ratio in GSD patients, it appeared that the (E3) allele's frequency was declining at the expense of the (E4) allele. This data raises the possibility that (E3) alleles are protective and (E4) alleles are predisposing. When compared to the (E2) and (E3) alleles, the apolipoprotein (E4) allele dramatically increased the risk of gallstone disease in an APOE genotypic model [34].

Sample size, research methodology, as well as genetic and environmental variables unique to each community, are some possible explanations for these variations. Also, failure to identify APOE2/2 and APOE4/4 is most likely caused by the limited sample of participants and uncommon frequency of each allele in this community under study.

Individuals with the (E2)and (E2) allele showed decreased cholesterol absorption and a high rate of bile salt production, suggesting that the protective effect of the (E2)allele in cholelithiasis may be in the metabolic pathways causing cholesterol supersaturation [35]. Additionally, it is hypothesized that people carrying the (E2)and (E2) alleles have long nucleation times for gall bladder bile while people with the (E4) allele have short nucleation times for gallstone formation [36], despite the fact that not all studies have seen this effect [37].

Due to changing in receptor affinity, APOE may inevitably affect the hepatic metabolism of cholesterol by enhancing cholesteryl ester hydrolysis[38] increasing the quantity of free cholesterol that is accessible to the biliary system within the cell. Additionally, there is proof that compared to (E3), (E4) causes internalized triglyceride-rich particle cholesteryl ester to produce more free cholesterol into the cell [11, 39] .

In present study we didn't found a relationship between the APOE4 allele and gallstone disease. Variation from population to population may be the cause of inconsistent results seen in different studies.

The substantial difference in mean serum TC levels was identified in GSD patients when compared to the control groups; these findings were consistent with prior research that found a positive link between serum TC levels and gallstones sufferers [40, 41]. On the other hand, some investigators stated no significant differences of serum TC were found between control and GSD patients [42, 43]. Gallstones can develop when bile becomes saturated with cholesterol, although the relation between gallstones and high TC levels in subjects is controversial and may be due to factors including heredity, socioeconomic status, and diet [44]. In this papulation the high level of cholesterol may be due to other factors such as consuming high calorie intake, sedentary life style.

The mean serum triglyceride level in the present study was higher in GSD patients than in controls, although the differences were not statistically significant. A similar study found that the differences in mean blood triglyceride levels between control and patient groups were not statistically significant [42] As opposed to the results above, it was discovered that patients with gallstones had considerably higher triglyceride levels than the control group [24, 41]. Some confirmations are that triglycerides in bile reduce gallbladder motility, which leads to gallstone formation. Patients had substantially greater mean serum HDL levels than control groups. Similar results have been reported by previous studies were in line with these results [44, 45]. this result disagreed with the previous study [43]. However, some studies have found no connection between HDL levels and gallstone development. In other words, HDL cholesterol is the primary source of biliary cholesterol[46].

In the current investigation, mean serum LDL and levels were significantly higher in GSD groups than control groups. Some studies were confirmed the same results [40, 43]. These findings contradict previous study that found a negative relationship between serum LDL levels and cholelithiasis [42]. Sample size, study methodology, and genetic and environmental factors unique to each community are possible explanations for these variations.

In the present study, the frequency of genotypes and allele, comparing between control and patient groups were not statistically significant (Table 4), Similarly, no significant differences were found according to genotypes (Table 5). A study conclude that LDL and total cholesterol levels are frequently higher in (E4) allele carriers who are homozygotic (E4/4) or heterozygotic (E4/2 or E4/3) [33]. As a result, having one (E4) allele causes an increase in chylomicron absorption and a reduction in LDL receptor expression, which may lead to a drop-in bile salt production and a decrease in salt secretion rates by this increase cholesterol crystals

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in the bile and more stones with a higher cholesterol content [47]. whereas other studies found no connection at all between hyperlipidemia and gallstones [33, 48]. The APOE protein can control the absorption of chylomicrons and VLDL-residual particles from the circulation through particular receptors, made possible by lipid-binding and receptor-binding domains. The polymorphism alters how well APOE binds to lipids and the LDLR, which has a variety of consequences on lipid and cholesterol metabolism and risks for several health problems [49]. Our study's findings revealed no correlation between the SNPs under investigation and either the likelihood of developing gallstone disease or the makeup of gallstones. A complex condition, gallstone disease is influenced by numerous variables. Its growth is influenced by environmental and genetic factors. diet, hormonal issues, particularly those involving sex hormones, obesity, diabetes, lipid metabolism issues, and medicine all play a significant effect. Genetic polymorphisms seem to have a minimal effect; nonetheless, they must be considered along with other environmental risk factors.

Conclusions

The study concludes that, these findings showed no correlation between the investigated polymorphisms and cholelithiasis. However, (APOE4) seems to have no or very little correlation with gallbladder stone formation. (APOE3) was also suggested to play protective roles, but further research with larger sample sizes is needed to corroborate this claim. Additionally, the study indicates a favorable relationship between cholelithiasis and lipid profiles. High levels of TC, LDL, and VLDL are risk factors, whereas high levels of HDL are protective.

Recommendations

To find the genetic markers that identify groups at risk and to develop new preventive and therapeutic approaches, similar studies from other communities should be supported.

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Conflicts of Interest

Nill

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Ethics Statements

The investigation was done under the supervision of Garmian University, College of Education, Department of Biology, ethics committee (No. 8: June 20, 2021), which followed the Declaration of Helsinki.

Author Contribution

The authors confirm contribution to the paper as follows: study conception and design: Ismail Salih Kakey; Data collection, analysis and interpretation of results and draft manuscript preparation: Seerwan Assi Raheem. All authors reviewed the results and approved the final version of the manuscript.

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