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Original Article

The impact of celiac disease on lipid profile parameters among patients with gallstones disease

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Abstract

Celiac disease is an autoimmune disease in genetically susceptible patients upon gluten-rich food intake, and it is thought to have an impact on serum lipid parameters in gallstone patients. The aim is to determine if celiac disease affects serum lipid parameters among patients with gallstone disease. A total of 280 people were included in this prospective study; 134 had gallstone disease, 6 patients had celiac and gallstone disease, and 12 asymptomatic celiac patients were involved. All participants were subjected to immunological investigations (anti-gliadin IgA and IgG; anti-transglutaminase IgA and IgG) via ELISA (Enzyme-Linked Immunosorbent Assay) technique. Biochemical investigations (Lipid profile test) were performed to check serum lipid parameter fluctuations. The gallstones disease and active celiac disease case's mean age was 41.01 years. Six patients revealed a positive ultrasonography exam for gallstones and a positive immunological test, including anti-gliadin IgA, IgG, and anti-transglutaminase IgA (greater than 10 U/ml). The asymptomatic or silent celiac disease group comprised 12 healthy persons with seropositive immunological results and still with silent symptoms of celiac disease with positive anti-gliadin IgA and IgG only (greater than 10 U/ml). Furthermore, the present research revealed that these two diseases substantially or relatively significantly influenced total cholesterol, triglyceride, LDL, and HDL. Active celiac disease influences serum lipid parameters due to interference with intestinal absorption via intestinal dysmotility and affecting neuropeptides via villi atrophy and crypt hyperplasia. Serum lipids disturbance and biliary cholesterol supersaturation due to intestinal absorption defect in active celiac patients make together to gallstones formation.

Keywords: celiac disease, gallstones disease, cholelithiasis, cholecystokinin, liver function test.

Introduction

Celiac disease (CD) is an autoimmune enteropathy defined by a lifelong sensitivity to eating gluten in people genetically predisposed to it [1]. CD prevalence may affect one in every 100 persons worldwide [2, 3]. An abnormal immunological reaction to gluten may cause intestinal enterocyte inflammation and damage [4]. Immunological response in genetically susceptible persons will appear, resulting in humoral immunity activation and autoimmune antibody production [5]. In recent years, a number of CD patients had normal or elevated body mass index (BMI) [6].

Respectively, gallstones disease (GD) is a hepatobiliary disorder defined by the formation and development of gallstones in the hepatic bile duct (HBD), common bile duct (CBD), or gallbladder (GB) as a result of bad cholesterol, bilirubin, and bile acid metabolism [7]. It is a serious public health issue in Europe and other affluent nations, affecting up to 20% of the community [8]. Patients with celiac disease have decreased postprandial GB emptying due to a significant reduction in sufficient neuropeptide hormone cholecystokinin (CCK) secretion from the atrophic small bowel mucosa, as proven by low CCK levels in both duodenal extracts and plasma [9]. Cholesterol-supersaturated bile has a

prolonged residence in the GB lumen, allowing for fast cholesterol crystallization and crystal development of macroscopic and microscopic gallstones due to CCK insufficiency. It also increases short bowel transit times and these changes improve cholesterol uptake efficiency as well as enhance intestine-derived cholesterol for biliary hypersecretion, resulting in cholesterol-supersaturated bile [1]. Gallstone disease has an estimated frequency of 5 to 22% [10]. Gallstones form as a result of a bile chemical imbalance caused by numerous processes that cause bile to be lithogenic. In the lithogenic condition, cholesterol levels can reach 8–10%, with increased biliary production of cholesterol being the most important contributing component. This might be due to a high-calorie, high-cholesterol diet, obesity, or enhanced activity of the hydroxy methyl glutaryl (HMG) coenzyme A reductase enzyme, which limits the rate of hepatic cholesterol production and increase hepatic absorption of cholesterol from the circulation [11]. Triglyceride level elevation was also linked to a slightly elevated risk of gallbladder stone formation or sludge incidence, which could be explained by the coexistence of TG level elevation and obesity. Low-density lipoprotein (LDL) elevation level has been associated significantly with gallstone disease [12].

Serologic tests are considered a gold standard in the diagnosis of celiac disease, which includes anti-gliadin IgA (G/IgA), anti-gliadin IgG (G/IgG), and anti-tissue transglutaminase IgA (T/IgA) and anti-tissue transglutaminase IgG (T/IgG). Anti-transglutaminase IgA test is essential because of its high specificity and sensitivity and its levels correlate with the severity of intestinal damage [13]. In celiac disease (CD), the loss of probiotic species (Lactobacillus and Bifidobacterium) and the relative growth of pro-inflammatory bacteria (Veillonaceae genera) are microbiota fingerprints that are likely contributing to disease development. A gluten-free diet (GFD) was shown to exert a positive effect via lowering bacterial richness while changing gut microbiota composition differently based on health and disease status CD in all groups studied [14]. Probiotics have been demonstrated to hydrolyze immunogenic gluten peptides, lowering their immunogenicity.

Furthermore, gut bacteria have been linked to immune response modulation, influencing the optimal development of anti-inflammatory Treg cells. Circulating T cells were obtained from CD patients who had an oral wheat challenge, and there was an increase in both effector T cells and Treg FOXP3+ cells; however, these FOXP3+ T cells had considerably lower suppressive activity. Villous shortening in a small intestinal wall is caused by a mix of innate and adaptive immunological responses [15].

Several CD-related characteristics in the duodenum have historically been seen in upper gastrointestinal endoscopy, including reduced mucosal (Kerckring) folds, scalloping and fissuring of the mucosa, mosaic pattern, and nodularity [16]. Disrupted gallbladder contractility and intestinal CCK hyposecretion have been observed to dramatically promote the production of cholesterol gallstones in CD by affecting GB empty and bile cholesterol metabolism and boosting intestinal cholesterol absorption. Individuals with pigment gallstones have a mild GB motility problem with no GB inflammation and an enlarged fasting GB [17].

Fraquelli *et al*. 2003, stated the impact of CD on gallstone formation via reduced postprandial enteric peptide secretions and increased gallbladder volume. GB motility has been investigated in untreated celiac patients, and the result confirms that decreased CCK secretion will affect intestinal and gallbladder motility [18]. A diet with low-calorie content may induce GB stasis and, consequently, a reduction in GB stimulation. Fat intake plays a crucial role in inducing GB contraction; this idea was introduced by Gebhard *et al*. 1996 [19].

Additionally, GB stasis promotes the conversion of cholesterol into cholesteryl esters for deposition in the GB wall, exacerbating the already poor GB motor function. More specifically, a longer retention period of cholesterol supersaturated bile inside the biliary lumen commonly results in rapid cholesterol crystal development, crystallization, and aggregation as micro lithiasis, and subsequently macroscopic gallstones, not just in CD patients but also in non-celiac individuals [20]. Most importantly, in celiac patients, biliary cholesterol synthesis and secretion are substantially doubled, indicating an increase in biliary cholesterol secretion is a crucial component in the formation of supersaturated bile. The chylomicron remnant pathway delivers a significant fraction of the cholesterol particles absorbed from the intestinal tract to the liver enabling hepatic hypersecretion into bile. This helps to explain why untreated celiac patients have excessive hepatic cholesterol secretion [21]. Researchers who had examined GSD and GB motility in CD cases via studies or clinical surveys did not investigate the impact of these diseases on lipid profiles in celiac and non-celiac cases with gallstones. Also, none of the researchers deal with his study investigation of CD in gallstone patients; therefore, a gap appears in previous studies and researches. In this study, we will cover this gap by investigating gallstone patients, checking the effect of

gallstone disease on lipid profile alone, and the impact of CD and GSD combined.

Material and methods

Study participants

This prospective study has conducted on 280 participants (aged ≥15 years) in Basrah Teaching Hospital from December 2021 to August 2022. A total of 140 patients were admitted into the surgical world, diagnosed, examined, investigated, and followed up. They underwent abdominal ultrasonography and the results indicated that they were diagnosed with gallstones and have prepared to perform laparoscopic cholecystectomy. Six patients among them had celiac as well as gallstones disease. They have seropositive test results of anti-gliadin autoantibodies IgA, IgG, and anti-transglutaminase autoantibodies IgA. About 140 healthy control subjects were chosen randomly from the outpatient clinic, those who visited the outpatient clinic for general health checkups. All of them were checked by an ultrasonography consultant to exclude GSD. Immunological and biochemical tests were performed on healthy persons to rule out CD and compare the results to the GSD group. Twelve patients had anti-gliadin IgA seropositive biomarkers result and clinical presentations indicated the patients were asymptomatic celiac patients. All 280 participants were subjected to a serum lipid profile test.

Study design

Patients with gallstones and asymptomatic celiac disease who were to be healthy were assessed by questionnaire. Blood samples collected in this study were obtained from fasting patients for 12 hours at least. Four milliliters of fresh vein blood sample was taken by a laboratory technician using a sterilized syringe. Collected blood samples were put into a specific GEL tube (acid citrate dextrose) that helps the blood sample to coagulate rapidly and enhances the separation of the sample into two phases after centrifuging them. After 20 minutes, the Centrifuge process was conducted using an electrical centrifuge instrument for 5 minutes at a speed of 4000 rpm. Beyond centrifuging, we obtain serum samples using a micropipette and put them into Eppendorf tubes, each tube containing (0.5 ml) and ready for preservation. The samples were stored immediately and transported directly into a deep-freezing instrument at the BASRA BIOBANK to prevent serum sample damage. Samples were kept at -50°C, ensuring the biomarkers and serum protein content were still undegraded and kept these contents active for 6 months [22]. Serum samples were used to achieve the celiac biomarkers: (anti-tissue transglutaminase T/IgA and T/IgG, anti-gliadin-A IgA, and anti-gliadin-G IgG) and serum lipid profile.

Immunological markers of celiac disease

Anti-gliadin markers IgA and IgG were detected via indirect ELISA (Demeditec Diagnostic GmbH (Germany), cut off value for both isotypes markers was (10 U/ml).

Anti-tissue transglutaminase markers IgA and IgG were detected via indirect ELISA (Demeditec Diagnostic GmbH (Germany), cut off value for both isotypes markers was (10 U/ml).

For a quantitative examination, the absorbances of both the standards as well as controls are graphed *versus* their levels. The level values for each specimen can then be obtained from the resulting reference curve concerning their absorption spectra. It is also feasible to employ computer applications that run automatically [23, 24].

Biochemical markers of serum lipid profile

Using a commercial kit from Linear Chemicals – Spain, blood samples were tested for total serum cholesterol, triglycerides (TG), High-Density Lipoprotein (HDL), and Low-Density Lipoprotein (LDL). Total cholesterol value represents the summation of the High-Density Lipoprotein (HDL) value, Low-Density Lipoprotein (LDL) value, and 20% of triglyceride (TG) value [25]. All of these tests depend on the measured absorbance (A) of both the samples and standard compared to the reagent blank.

Statistical analysis

The data were analyzed using the statistical software SPSS-26.0 (SPSS Inc, Chicago, IL). Quantitative data were represented using basic measurements of mean and standard deviation. The significance of differences between means was assessed using the ANOVA test for differences between more than two independent means, followed by the Tukey test. Chi [26] was used to investigate any association between qualitative variables. Statistical significance was regarded

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when the P value was less than 0.05 and highly significant when it was less than 0.01.

Results

Demographic findings

The demographic findings of participants enrolled in this study showed 134 patients (14 male and 120 female) with GSD only without the CD of ages range (19–85 years) with a mean value (41.01) who have claimed clinical features of gallstones, 6 female patients of ages range (15–52) years with a mean of (34.33) revealed seropositivity of anti-gliadin and anti-transglutaminase autoimmune antibodies in a varying degree and appeared as overt CD in addition to GSD. A number of 128 healthy control persons (14 male and 114 female) who did not complain of any disease, age range (16–76) years with a mean of (40.99). Twelve healthy female persons with a seropositive immunological result and still with silent symptoms of CD, ages range (17–68) years, with a mean of (39.83) (Figure 1).

Our study has shown the female/male ratio for gallstone prevalence was 9:1. Residence distribution of study groups indicated that most of the patients and healthy control were of urban residence and a small percentage were of rural residence. The residence of patients in the GSD group was distributed as (120, 89.6%) urban and (14, 10.4%) rural. The patient's residence in the dual disease group was distributed as (6, 100%) urban. The distribution of residence in the healthy control group was (106, 82.8%) urban and (22, 17.2%) rural, and finally, the distribution of residence in the asymptomatic Celiac disease group was (7, 58.3%) urban and (5, 41.7%) rural (Table 1).

Gliadin autoantibody G/IgA

Gliadin autoantibody G/IgA showed highly significant differences in G/IgA titers (P<0.01) among the study groups. The dual disease group showed the highest mean value (85.58 U/ml±128.05) greater than the upper limits (≥10 U/ml), indicating the people in this group have CD with GSD. The healthy control group revealed the lowest mean value (2.35 U/ml±1.39). The GSD group showed G/IgA with a mean value (2.63 U/ml ±1.57) and the CD group is mean value (17.4 U/ml±8.74) (Figure 2 A).

Gliadin autoantibody G/IgG

Gliadin-specific antibody marker (G/IgG) findings among study groups revealed a significant difference

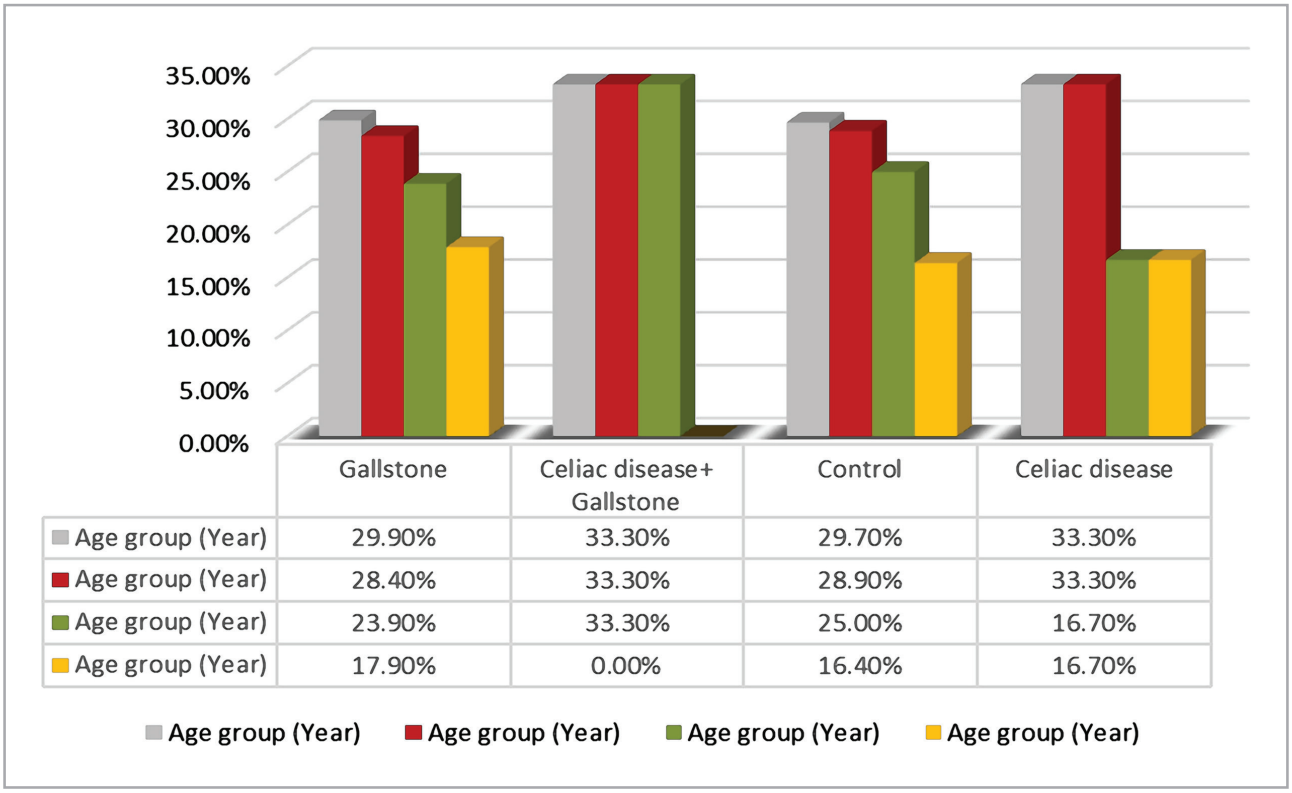

Figure 1: Study groups stratified by age.

Table 1: Variables of the study.

Variables		Category				Total
		Gallstone disease	Silent celiac disease	Celiac disease + gallstone	Control	
Sex	Male	14	-	-	14	28
		10.4%	-	-	10.9%	10.0%
	Female	120	12	6	114	252
		89.6%	100.0%	100.0%	89.1%	90.0%
Residence	Urban	120	7	6	106	239
		89.6%	58.3%	100.0%	82.8%	85.4%
	Rural	14	5	-	22	41
		10.4%	41.7%	-	17.2%	14.6%
Total		134	12	6	128	280
		100.0%	100.0%	100.0%	100.0%	100.0%

(P<0.01). The dual disease group showed a mean value (36.91 U/ml±20.87) greater than the upper limits (≥10 U/ml), indicating that these patients have celiac disease (CD) with Gallstone disease (GSD). The GSD group showed G/IgG with a mean value of (2.68 U/ml ±1.65), the healthy control group revealed the lowest mean value (2.37 U/ml±1.56), and the CD group showed a mean value (of 13.75 U/ml±11.06) with a slight elevation more than upper limits (≥10 U/ml) (Figure 2 B).

Transglutaminase autoantibody T/IgA

Transglutaminase-specific antibody marker (T/IgA) results revealed a highly significant difference (P<0.01) between study groups. The dual disease group showed the highest mean value (74.85 U/ml±148.55) greater than the upper limits (≥10 U/ml), indicating the people in this group have CD with GSD. GSD group showed the lowest mean value of tTG/IgA (2.05 U/ml±1.40) and the silent CD group showed a mean value of (8.73 U/ml ±8.09), subsequently, the healthy control group showed a mean value of (2.46 U/ml±1.46) which stays below upper limits of normal values (Figure 3 A).

Transglutaminase autoantibody T/IgG

The results of (T/IgG) transglutaminase-specific antibody markers revealed a highly significant difference (P<0.01) between study groups. The dual disease group showed a mean range greater than 7 U/ml, indicating that the T/IgG titer is slightly elevated, above the normal range below 7 U/m. GSD group revealed T/IgG with a mean value of (2.06 U/ml±1.34), the dual disease group showed a mean value (of 8.61 U/ml±10.30), the control subjects group showed a mean value (of 2.36 U/ml±1.45), and asymptomatic Celiac disease group revealed mean value (6.40 U/ml±8.36). Also, the dual disease group showed the highest mean value (8.61 U/ml±10.30), while the GSD group revealed the lowest mean value (2.06 U/ml ±1.34) (Figure 3 B).

Cholesterol

The gallstones disease group averaged (202.88 mg/dL ±47.20), the dual disease group averaged (174.50 mg/dL ±27.49), the control subjects' group averaged (194.86 mg/dL ±48.26), and the silent celiac disease group averaged (190.50 mg/dL±53.27). The lipid profile data of the research groups, including cholesterol, were compared and revealed insignificant changes (P>0.05) between them. The dual disease group had the lowest mean value (174.50 mg/dL±27.49), whereas GSD had the highest (202.88 mg/dL±47.204) (Figure 4 A).

Triglycerides

The GSD group averaged (140.92 mg/dL±79.54), the dual disease group averaged (99.33 mg/dL±21.15), the control participants' group averaged (168.70 mg/dL ±106.07), and the silent celiac disease group averaged (144.17 mg/dL±85.60). When TG levels were compared across study groups, there was a significant variation (P<0.05) between them. The dual disease had the lowest mean value (99.33 mg/dL±21.15), whereas the healthy

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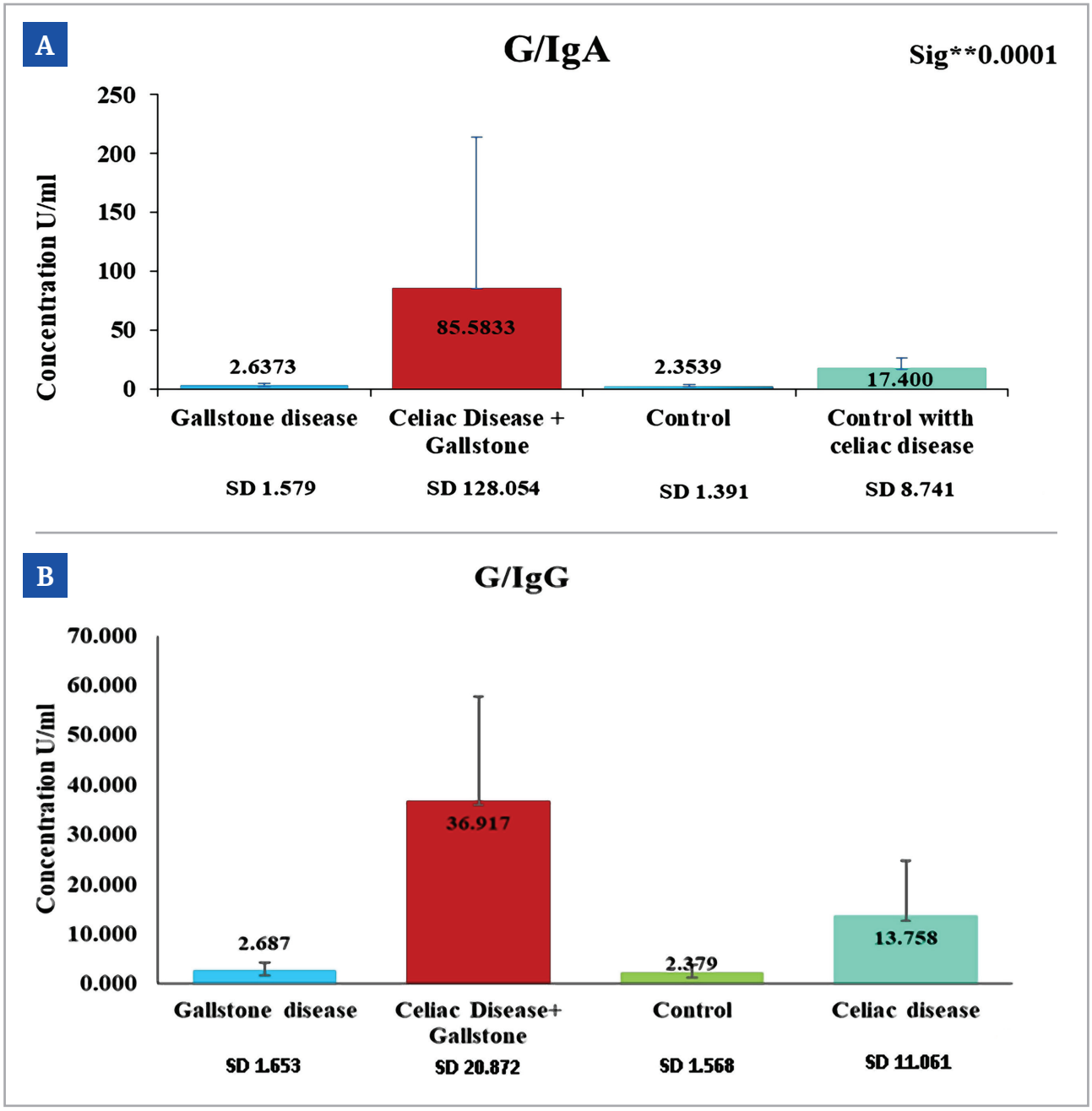

Figure 2: A – Level of G/IgA (U/ml) among study groups; B – Level of G/IgG (U/ml) among study groups.

control disease had the highest (168.70 mg/dL±106.07) (Figure 4 B).

High-density lipoprotein (HDL)

The GSD group revealed HDL with a mean value of (44.57 mg/dL±8.22), the dual disease group showed (41.00 mg/dL±4.336), the control subjects group showed (46.46 mg/dL±8.41), and asymptomatic celiac disease group revealed (49.33 mg/dL±11.428. There were significant variations ($P<0.05$) across research groups when comparing HDL values. The asymptomatic CD group showed the highest mean value (49.33 mg/dL±11.42), while the Celiac plus Gallstones disease group revealed the lowest mean value (41.00 mg/dL±4.33) (Figure 4 C).

Low-density lipoprotein (LDL)

The GSD group averaged (131.57 mg/dL±39.40), the dual disease group averaged (104.83 mg/dL±33.51), the control patients' group averaged (117.38 mg/dL ±39.88), and the silent celiac disease group averaged (114.75 mg/dL ±39.07).

Comparing the LDL levels among the research groups revealed a significant difference ($P<0.05$). Celiac plus Gallstones disease had the lowest mean value

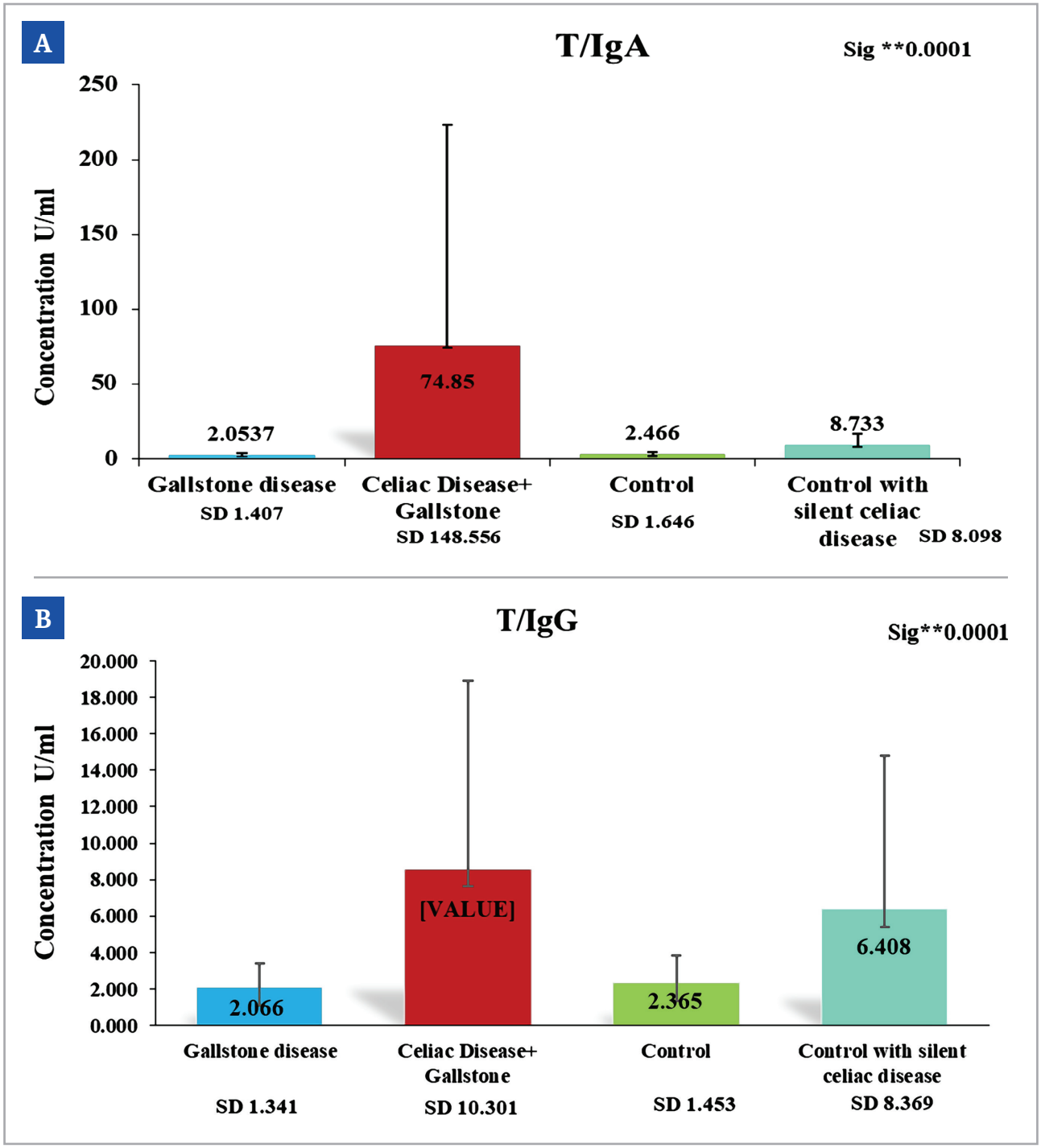

Figure 3: A – Level of T/IgA (U/ml) among study groups; B – Level of T/IgG (U/ml) among study groups.

(104.83 mg/dL±33.51), whereas GSD had the highest (131.57 mg/dL±39.40) (Figure 4 D).

Discussion

Age is considered a predisposing factor to the risk of celiac disease and gallstone incidence [27–29]. The results of the present study revealed that age is closely related to GSD occurrence and shows high frequency in patients younger than 50 years, and the number of patients decreased in older age. However, we noticed in our study that all patients have an equal chance of getting CD regardless the age. This may be related to the small population of the CD group [30, 31].

Some studies have proven different results; disease prevalence increases with age, especially over 60 years old, with a positive association between GSD trend and age has been revealed [32]. Age substantially affects other variables in this study, and we will highlight the

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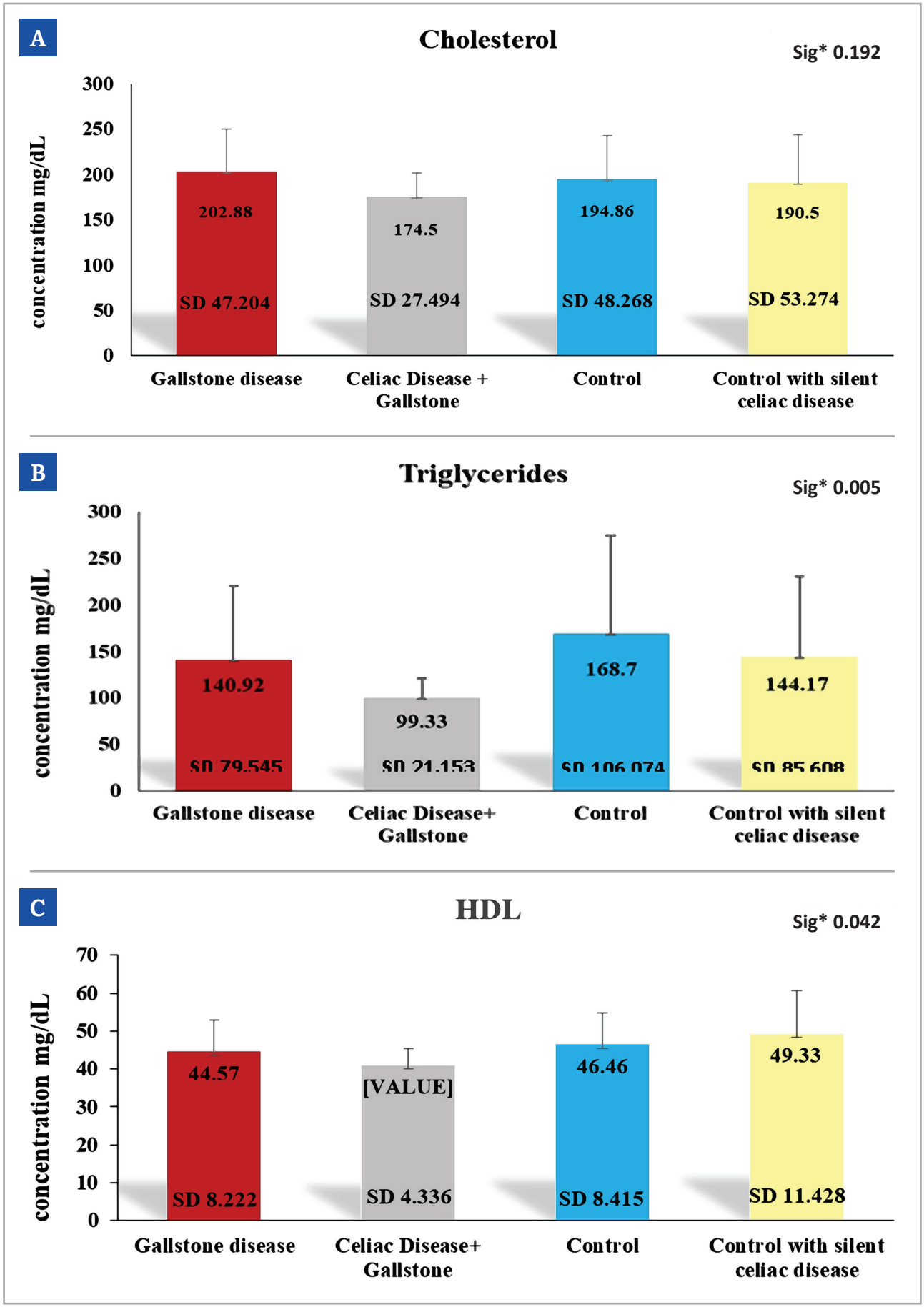

Figure 4: A – Level of cholesterol (mg/dl) among study groups; B – Level of triglyceride (mg/dl) among study groups, C – Level of HDL (mg/dl) among study groups; D – Level of LDL (mg/dl) among study groups.

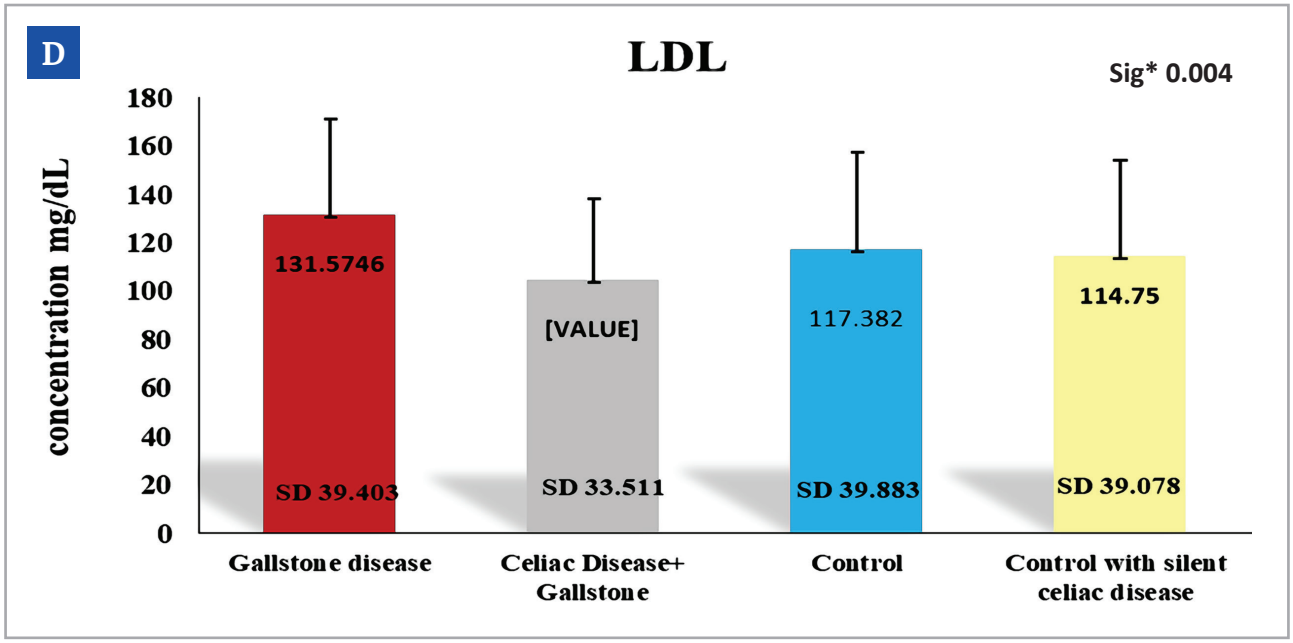

Figure 4: Continued.

impact on these variables. To begin with, our study showed that age in the gallstone group has a significant positive relation (p=0.001), (R=0.275) with total serum cholesterol (CHOL), furthermore it has a significant positive relation (p=0.00), (R=0.398) with triglycerides (TG) and in conclusion, it has a significant positive relation (p=0.009), (R=0.22) with LDL. In other meaning, age progression may cause obesity and BMI elevation as well as metabolic syndrome, which act together to increase the tendency of gallstone formation.

Age as a risk factor in the population has little effect on CD incidence; however, the children population showed a significant disease occurrence relative to adults [33, 34]. CD patients frequently have a nutritional impairment, with lower body weight including fat-free content, dietary inadequacies, and unbalanced blood lipids, such as low HDL level due to decreased synthesis and secretion of (APO-A1) lipotropin, significant elevation of TG and total serum cholesterol and these modifications may continue even after GFD therapy [35].

In the present study, all age groups have an equal chance of getting a CD and no correlation between age and disease; consequently, the effect of age may be related to metabolic disorders which synergize with CD. Moreover, CD occurred equally in all age groups approximately and decreased or was not detected in old ages more than 60 years old.

Demographic parameters represented by the sex variation, the commonest sex that prone to have GSD is female, and the incidence in females more than in males due to increased bile cholesterol excretion and endogenous synthesis via female reproductive hormones that can induce gallstone development, as demonstrated by animal research and recently, testosterone has been linked to gallstone production in men. The number of births can increase the chance of getting gallstones [36]. Sex significantly influences gallstone occurrence, as females have a higher frequency than males. Hormonal changes in the female have one of the important factors that cause the formation of gallstones. This is in acceptance of our findings. GSD hazard was associated with TG in females while not in males, which is consistent with prior findings.

Additionally, cholesterol, HDL cholesterol, as well as LDL cholesterol were found to have larger preventive advantages in women than that in men. As a result, estrogens may increase the probability of GSD by enhancing the hepatic production of biliary cholesterol, which increases bile cholesterol saturation [37]. The undiagnosed CD is more common in women than in men and the increased risk of CD for female participants compared to male people involved is greater in children than in adults [38].

By reviewing the previous results, we noticed sex as a predisposing factor plays a crucial role in GSD incidence, and the sex effect on CD is difficult to admit because the number in this group was only 6 patients and the healthy control group gave rise to only 12 asymptomatic patients. The ratio of females/males in the healthy group was selected according to the ratio of females/males in the GSD group.

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By rechecking the residence findings in demographic results, 13 patients with CD, seven patients (58.3%) with asymptomatic CD, and six patients with active CD reside in urban regions *versus* only five asymptomatic patients (41.7%) residing in rural regions. Also, 126 (89.6%) patients with GSD reside in urban regions *versus* only 14 (10.4%) in rural regions.

Patients with GSD in rural locations are frequently underserved and cannot have the same access as their urban counterparts, particularly to health care. It was proposed that greater engagement in a Modern lifestyle, particularly increasing saturated fat with decreased fiber consumption, might be a potential factor for this. Increased BMI, in conjunction with increased urbanization, is a significant potential factor in the genesis of GSD [39]. Rural populations have more disparities in health care because it has been founded that individuals who live in rural regions would be less likely to have healthy meal retail outlets and grocery stores, so it may be a requirement that comes with rural regions and is therefore not as widely reported as a restriction for such areas compared to urban areas [40].

The effect of residence on the disease was reviewed by previous studies [39–41] and these were in agreement with our study findings; it could reflect the impact of the environment, nature of life, maybe nature of nutrition, and the level of education via the direct influence of these factors on disease discovering or progressing.

Although Anti-Gliadin Antibodies (AGA) tests historically have been useful in CD diagnosis, it is no longer commonly recommended due to their lower specificity for CD diagnosis compared to other specific tests such as anti-tissue transglutaminase [41]. Serology-alone diagnosis is becoming more prevalent, and European pediatric guidelines outline requirements for an intestinal biopsy-free serological diagnostic method [42]. Several clinical studies have found that positive anti-gliadin antibodies IgA (G/IgA) is the first diagnostic marker of gliadin enteric sensitivity and progression of the CD. Alternatively, because autoantibody AGA has a high sensitivity and a low specificity for diagnosing CD, this finding can be explained by the presence of AGA in healthy people. Furthermore, AGA is positive not only in CD but also in neuropathy disorders, as well as other autoimmune and psychiatric disorders [43]. Celiac-specific antibodies are created in the intestinal mucosa of patients after gluten exposure and seem to be within diseased intestinal epithelium, saliva, and blood [41]. The severe elevation of the mean value of G/IgA titers in the dual disease group results from an immune response upon gluten exposure and the occurrence of a positive anti-gliadin autoantibodies AGA. G/IgA most likely indicates CD false seropositivity if negative anti-transglutaminase antibodies T/IgA [44]. Recent evidence and updated guidelines for adults can no longer support biopsies across all individuals genetically predisposed to CD and have been diagnosed by serological testing with symptoms and clinical signs of CD [45].

The findings of G/IgA indicated the patients in the dual disease group have CD, while the asymptomatic CD group showed false seropositive results of gliadin autoantibody G/IgA due to negative result of T/IgA and this explained why patients in this group were asymptomatic. Another explanation for the asymptomatic celiac group is that they have slightly elevated titer and are still asymptomatic; patients in this group may have had the disease recently and need more titer elevation or have gluten sensitivity without symptoms. By focusing on the mentioned results, the elevation of anti-gliadin antibodies G/IgA, multi foods more than its upper limits may precipitate GSD as a complication of CD.

Deamidated gliadin peptide-IgG (DGP-IgG) test is frequently used for CD screening. Tissue transglutaminase-IgA antibody is more sensitive and selective than DGP-IgG antibody. DGP-IgG is a sensitive method for CD discovery, especially in IgA-deficient people. A positive DGP-IgG with the presence of a negative T/IgA has low diagnostic accuracy for CD in children, although it can be increased in several non-celiac gastrointestinal diseases for unknown causes, conjunction with digestive problems or biochemical signs of malabsorption or inflammation [46]. Positive G/IgG is difficult to be interpreted, especially with negative T/IgA, an endoscopic duodenal biopsy was performed on forty individuals who tested positive for gliadin peptide G/IgG but negative for T/IgA; only one of those patients had CD verified by biopsy, and this patient lacked IgA antibodies. Regarding separated G/IgG positive serology, this results in a positive predictive value of about 2.5% [47]. These studies were in agreement with our study findings.

The G/IgG assay did not affect the diagnostic performance of the T/IgA test. The serology of T/IgA was favorably linked both for IgG and IgA gliadin peptide antibodies, with a signed agreement with T/IgA and G/IgG and moderate correlation with G/IgA. G/IgG, but not IgA, was equivalent to T/IgA, indicating that it might be used as a reliable option for CD diagnosis and follow-up in case of T/IgA was positive [48].

The silent CD group showed insignificant difference with a slight elevation of G/IgG titer with negative T/IgA that confirmed they are free of CD and they have either gluten sensitivity with non-inflammatory

changes or patients have recently the disease without enteropathy or intestinal damage and no presence of histological modification like villous atrophy and crypt hyperplasia. It needs more time for titer elevation and induces bowel damage, and makes the symptoms appear in these groups.

Previous research [46, 47] that explored the association between anti-gliadin IgA and IgG with gallstone occurrence revealed a negligible correlation between them. Many studies included GIT and liver disorders such as IBS but did not include this correlation. Anti-gliadin autoantibodies (AGA) might be detected in non-celiac gluten sensitivity cases and considered indicators of gluten sensitivity without giving any image about intestinal damage and enteropathy. GSD occurrence is closely related to intestinal dysmotility, intestinal enteropathy, and villous atrophy due to CD and CCK neuropeptide secretion disturbance [49].

Strong evidence supports the utilization of T-IgA assays as a first-line test for CD diagnosis. This assay has a (95%) sensitivity and specificity in identifying people with CD if the individual is on gluten-containing nutrition at the time of testing [50]. The CD is recognized by the production of sera-specific autoantibodies (*e.g.*, T/IgA), positive titers that indicate small intestine mucosa damage, and various clinical pictures [51].

Elevated specific and sensitive serum antibodies and IgA-tissue transglutaminase (tTG) are used as preliminary and primary diagnostic markers. There is a link between T/IgA production as well as the severity of intestinal mucosal alterations. In IgA-deficient individuals with positive T/IgG serology, no such association was detected [40]. Although T/IgA positive has long been regarded as the gold standard for CD diagnosis, several facilities have incorporated at least one further serologic test into the celiac screen to increase screening sensitivity. The occurrence of negative anti-transglutaminase antibodies T/IgA and positive anti-gliadin antibodies AGA IgA is most likely indicative of CD false positivity. Elevation of anti-tissue transglutaminase antibodies T/IgA several folds in the dual disease group, higher than its normal upper limits, indicating patients in this group have CD. In contrast, the asymptomatic Celiac disease group showed seronegative results of tTG T/IgA, which explained why patients in this group were asymptomatic [44]. Autoantibodies may precipitate GSD as a complication of CD.

Several studies have found a link between higher blood anti-TG2 blood levels and tissue damage. The researchers discovered that the greater the sera anti-TG2 antibody level, the more severe the histopathological intensity of the lesion in the small intestine. The existence of CD is the most critical factor influencing serum anti-TG2 antibody levels [52].

Cholesterol supersaturation leads to GB mucosal damage, and epithelial cells do not possess the ability to produce lipoproteins for excessive cholesterol amounts causing storage of cholesterol in epithelial cells and lamina propria of mucosa as cholesterol monomers and cholesterol ester that increase mucosal damage and lymphocytes infiltration and so on inflammation [53]. In hypercholesterolemia, gallbladder inflammation may be initiated via sphingolipids ceramide accumulation [54].

In a study from West Azerbaijan, mean cholesterol levels were considerably higher in celiac patients with gallstones than in those without gallstones [55]. Biliary flow rate, as well as bile component outflow, including cholesterol, phospholipids, as well as bile acids, were greatly elevated in an active CD before being normalized with effective GFD. This increased release of freshly generated and/or absorbed cholesterol immediately into the bile has been linked to a reduction in cholesterol levels in CD patients, as shown in both adult and pediatric clinical investigations. Plasma levels of cholesterol, in contrast, hand, are usually associated with a drop in HDL-cholesterol and, as a result, greater total cholesterol to HDL-cholesterol ratio, both of which can be addressed with GFD9. By looking at the study of Kharus *et al.*, we found low serum cholesterol in undiagnosed seropositivity celiac patients; these findings differed from the results obtained in our study [56]. Cholesterol decrease may be attributed to highly hepatic cholesterol secretion into biliary contents as a compensatory mechanism due to excessive absorbed cholesterol in Celiac patients in addition to endogenous cholesterol.

Total serum cholesterol did not affect by gallstones, celiac disease, or both. Total serum cholesterol levels are still within normal ranges or slightly elevated and are not indicative of differential cholesterol, so we measure other differential lipids in the blood, such as triglycerides, HDL, and LDL. These lipids have been used as an indicator to know the effect of diseases on lipid profiles.

Elevated TG levels may reduce sensitivity to CCK and enhance both biliary cholesterol saturation and bile viscosity by increasing mucin production, thereby increasing the risk of GSD. TG has an integral synergistic role with hypercholesterolemia to induce gallstone pathogenesis [54].

TG can stimulate the aggregation of cholesterol into microcrystals by promoting mucin production

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from GB mucosal cells [57]. Zhang *et al*. proved through their study that when individuals were stratified by age and gender, the rise in TG was linked with GSD risk [37]. CD may have a prominent role in TG dropping through its effect on intestinal absorption.

HDL revealed a close positive relation with cholesterol, with a high significance ($p<0.001$) in the GSD group, and also showed the same relation in a healthy control group, with a significant value ($p<0.001$). Otherwise, any increase in cholesterol leads to an increase in HDL and *vice versa*. In the dual disease group, HDL showed an inverse relation with TG, with a significant difference ($p=0.008$) that means an increase in TG leads to a decrease in HDL. This is thought to be the impact of CD in this group and may explain the decrease in total serum cholesterol and HDL while an increase in Triglycerides. Also, it is shown by the results that HDL in the Celiac plus Gallstones disease group had a dual effect of the two disorders that impacted HDL level and decreased it less than that in the healthy control group.

In our investigation, participants with GSD had reduced HDL cholesterol and greater TG, although the only difference between cases and controls was the incidence of higher TG. The link between HDL and GSD is yet unknown [58]. According to one study by Lauridsen *et al*., increased HDL levels were shown to be inversely related to gallstone prevalence, meaning that increased HDL values may be one of the pathways in the prevention and treatment of cholesterol gallstone illness [57, 59]. HDL, a bile acid precursor, is a significant source of biliary cholesterol; nevertheless, increased serum HDL enhances primary bile acid production, which helps break down cholesterol and lowers biliary cholesterol supersaturation, both of which are crucial for lowering bile lithogenicity [57]. Furthermore, HDL may facilitate the movement of excessive cholesterol from lipid-laden macrophages inside the arterial wall towards the liver for excretion into the bile, which is the primary pathway for irreversible cholesterol elimination from the body [54]. A high blood HDL cholesterol level has been shown to increase hepatic bile acid production and reduce the overall cholesterol saturation index, which increases cholesterol solubility within the bile and so protects against gallstone formation. It has also been demonstrated that HDL cholesterol accounts for the vast bulk of the cholesterol transported into bile. Given the negative connection shown in this study involving HDL cholesterol as well as GSD risk, it is plausible to assume that the free cholesterol within HDL is primarily transformed into bile acid instead of discharged into the bile as cholesterol [37].

These findings are not fitted to the results that we had found, where HDL level in the asymptomatic CD group is more than that of the healthy control group, this may be attributed to the small number population ($n=6$), which is one of the limitations in our study or due to reverse mechanism that led to increase HDL.

Serum LDL levels were elevated in GB cholesterolosis and served as a potential risk for GB cholesterolosis, likely to result in minor molecules of LDL infiltrating into the GB epithelium more rapidly, where its wall is densely implicated with macrophages, going to participate in the creation of foamy cells [54]. Additionally, Zhang *et al*. discovered that greater LDL cholesterol levels had substantially connected with GSD risk mostly in younger populations while inversely associated with GSD probability mostly in middle-aged and elderly populations in their investigation [37].

In the GSD group, LDL showed a significant ($P=0.00$) relation with cholesterol, TG, and HDL, meaning an increase in each one of them leads to a positive increase in LDL. There is a non-significant relation between LDL and another variable in the Celiac plus Gallstones disease group. In the asymptomatic CD group, LDL showed a significant relation with cholesterol ($p=0.001$). It is thought to be related to LDL inflammatory effect and macrophage infiltration.

Conclusion

Celiac disease is strongly sensitive to gallstones disease, and it is considered a risk factor by increasing the chance of having GSD via neuropeptide CCK disturbance, which interferes with gastrointestinal GIT motility and absorption. Gallstones disease has different strategies in celiac and non-celiac patients. In celiac patients, CCK theory is closer to reality, while in non-celiac patients, obesity, sex, and serum lipid disorder clearly affect getting GSD. GD potentially affected serum lipids such as cholesterol, TG, HDL, and LDL. We can distinguish between celiac and non-celiac gluten sensitivity by exclusion the immunological test. Further studies to add intestinal or duodenal biopsy to strengthen the diagnosis of the CD in immunologically negative persons are required. An invasive study of duodenal biopsy in any patient with suggestive symptoms of CD is of benefit.

Conflict of interest

The authors declare no conflict of interest.

Consent to participate

Written informed consent was obtained from the participants.

Ethics approval

The approval for this study was obtained from the Ethics Committee of the College of Pharmacy, University of Basrah (approval ID: 233).

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