Cukurova Medical Journal
ÇUKUROVA ÜNİVERSİTESİ TIP FAKÜLTESİ

Cukurova Med J 2022;47(4):1600-1609
DOI: 10.17826/cumj.1166923

ARAŞTIRMA / RESEARCH

Comparison of early atherosclerosis markers in children with Celiac disease and their healthy peers

Çölyak hastalığı olan çocuklar ile sağlıklı yaşıtlarının erken ateroskleroz belirteçlerinin karşılaştırılması

Derya Karpuz[1], Özlem Tezol[2], Merve Türkegün Şengül[3], Yusuf Usta[4]

[1]Mersin University School of Medicine Division of Pediatric Cardiology, [2]Department of Pediatrics, [3]Department of Biostatistics, [4]Division of Pediatric Gastroenterology, Hepatology and Nutrition, Mersin, Turkey

Cukurova Medical Journal 2022;47(4):1600-1609

Abstract

Purpose: We aimed to evaluate carotid intima-media thickness (cIMT) and epicardial adipose tissue thickness (EATT) concurrently as early atherosclerotic markers in pediatric patients with Celiac disease.

Materials and Methods: Patients with Celiac disease (n=54) and healthy peers (n=54) aged 5-18 years were enrolled in this cross-sectional study. Patients who followed gluten free diet at least the past 12 months were included. Anthropometric and biochemical measurements were performed. cIMT and EATT were measured by echocardiography and compared between the patient and control groups.

Results: Body mass index (17.4±3.0 vs. 18.4±3.1 kg/m^2), blood pressure (systolic: 100 (85-120) vs. 100 (80-100) mmHg; diastolic: 60 (40-90) vs. 70 (40-90) mmHg), and lipid profile (total cholesterol: 144.6±30.2 vs. 150.8±22.6 mg/dL; triglycerides: 71.5 (27-178) vs. 92.5 (34-203) mg/dL) were not different between the patient and control groups, while there were significant differences in cIMT and EATT. The patient group had higher cIMT (0.50±0.07 vs. 0.45±0.04 mm) and EATT (5.68±0.90 vs. 4.22±0.76 mm) than the control group. The risk of vitamin D insufficiency was 2.68 times higher in the patient group (95% CI=1.19-6.03).

Conclusions: Children with Celiac disease had higher cIMT and EATT than healthy peers. cIMT and/or EATT measurements by echocardiography may present as a reliable and easy method to investigate subclinical atherosclerosis in children with Celiac disease.

Keywords:. Atherosclerosis, carotid intima media thickness, celiac, children, epicardial adipose tissue thickness

Öz

Amaç: Bu çalışmada, pediatrik yaş grubundaki Çölyak hastalarının karotis intima-media kalınlığının (KİMK) ve epikardiyal yağ doku kalınlığının (EYDK) değerlendirilmesi amaçlandı.

Gereç ve Yöntem: Bu kesitsel çalışmada, 5-18 yaş aralığındaki Çölyak hastaları (n=54) ve sağlıklı yaşıtları (n=54) yer aldı. En az son 12 aydır glutensiz diyet uygulayan hastalar dahil edildi. Antropometrik ve biyokimyasal ölçümler yapıldı. Ekokardiyografi ile ölçülen KİMK ve EYDK değerleri hasta ve kontrol grupları arasında karşılaştırıldı.

Bulgular: Hasta ve kontrol gruplarında beden kitle indeksi (17,4±3,0'a karşı 18,4±3,1 kg/m^2), kan basıncı (sistolik: 100 (85-120)'e karşı 100 (80-100); diastolik: 60 (40-90)'a karşı 70 (40-90) mmHg) ve lipid profili (total kolesterol: 144,6±30,2'ye karşı 150,8±22,6; trigliserid: 71,5 (27-178)'e karşı 92,5 (34-203) mg/dL) farklılık göstermedi. Hasta grubunda KİMK ve EYDK, kontrol grubuna göre istatistiksel anlamlılıkta daha yüksek saptandı (KİMK: 0,50±0,07 mm'ye karşı 0,45±0,04 mm; EYDK: 5,68±0,90 mm'ye karşı 4,22±0,76 mm). D vitamini yetersizliği riski hasta grubunda 2,68 kat yüksek bulundu (%95 Güven Aralığı = 1,19-6,03).

Sonuç: Çölyak hastalığı olan çocukların sağlıklı yaşıtlarından daha yüksek KİMK ve EYDK değerlerine sahip olduğu görüldü. Ekokardiyografik KİMK ve/veya EYDK ölçümleri, Çölyak hastalığı olan çocuklarda subklinik aterosklerozu değerlendirmenin güvenilir ve basit bir yöntemi olabilir.

Anahtar kelimeler: Ateroskleroz, karotis intima-media kalınlığı, çölyak, çocuk, epikardiyal yağ doku kalınlığı

Yazışma Adresi/Address for Correspondence: Dr. Özlem Tezol, Mersin University School of Medicine Department of Pediatrics, E-mail: ozlemtezol@hotmail.com
Geliş tarihi/Received: 25.08.2022 Kabul tarihi/Accepted: 21.11.2022

INTRODUCTION

Celiac disease (CD) is a lifelong, immune-mediated, systemic disorder induced by gluten and related prolamins in genetically susceptible individuals, resulting in a variety of clinical manifestations and damage to intestinal mucosa[1]. The immunological reaction against gluten leads to histological abnormalities in the small intestine characterized by chronic inflammation, resulting in the development of villous atrophy and secondary malabsorption[2]. In CD, the impaired immune response does not seem to be limited to the small intestine and affects different organs with unknown mechanisms[3]. In adult patients with CD, both immune diseases and idiopathic cardiovascular diseases have been reported[4]. In connective tissue diseases, an increased risk for the development of early atherosclerosis and related complications has been demonstrated. It has been suggested that this increased risk is associated with systemic inflammation, and this association may explain etiology of atherosclerosis in all other chronic inflammatory diseases[5]. The risk of death due to cardiovascular diseases was found to be higher in patients with CD than in normal population[6]. An increased prevalence of CD was also reported in the presence of low serum high-density lipoprotein cholesterol, which is known to be a risk factor for cardiovascular diseases[7].

Carotid intima-media thickness (cIMT) and epicardial adipose tissue thickness (EATT) are accepted as early atherosclerotic markers and are correlated with cardiovascular morbidity and mortality[8,9]. It is important to recognize premature atherosclerosis before the onset of cardiovascular diseases which develop over the years. Increased cIMT can indicate the presence of subclinical atherosclerosis characterized by partially reversible endothelial dysfunction and remodelling of the arterial wall[8]. In pediatric CD patients, Demir et al. showed a correlation between tissue transglutaminase immunoglobulin A antibody (tTg-IgA) positivity and cIMT[10]. Based on the lower cIMT of children with a gluten-free diet compared to healthy children, the authors emphasized that a gluten-free diet can be protective against atherosclerosis[10]. Originating from the same embryological root as mesenteric and omental fat, epicardial adipose tissue is stored around the heart and coronary vessels and can produce inflammatory cytokines and free oxygen radicals that cause endothelial dysfunction. Therefore, EATT can reflect coronary atherosclerosis and myocardial function. Increased EATT is known to be a stronger coronary risk factor than increase in any other fat tissue in the body[9].

The atherosclerotic process begins in childhood and leads to ischemic complications over the years[11]. Therefore, in order to provide early interventions, it is important to identify children who potentially have an increased risk for cardiovascular diseases through adulthood. There is limited data on the development of early atherosclerosis in children with CD. The present study hypothesized that children with CD may have increased cIMT and/or EATT as early markers of atherosclerosis compared to healthy peers, and evaluated, for the first time, cIMT and EATT concurrently in pediatric CD patients.

MATERIALS AND METHODS

Study design and study sample

This comparative cross-sectional study carried out in a tertiary care university hospital and investigated the subclinical atherosclerosis of pediatric patients with CD. Celiac patients monitored in the Pediatric Gastroenterology, Hepatology and Nutrition department and healthy peers from well-child outpatient department of Mersin University Hospital were the subjects. The patients' and their mothers' written consents were obtained to participate. The study was carried out from June to September 2018 and Mersin University Clinical Research Ethics Committee approval was obtained (2017-09-21/256).

The minimum sample size was calculated as 54 subjects for the patient group and 54 subjects for the control group by comparing two means of cIMT in patients with CD receiving gluten withdrawal and healthy peers reported by De Marchi et al.[12], considering 95% confidence interval, 80% test power, and 1:1 ratio of sample size in "OpenEpi" program (https://www.openepi.com/SampleSize/SSMean.htm).

Data collection

Patients aged 5–18 years who diagnosed with CD confirmed by biopsy according to The European Society for Paediatric Gastroenterology Hepatology and Nutrition diagnosis criteria and followed gluten-free diet at least the past 12 months were included. Disease duration, histopathologic type according to modified Marsh-Oberhuber classification[13], and

biochemical findings were collected as clinical data. Children without any chronic disease and/or exclusion criteria constituted the control group. For both groups exclusion criteria were: younger than 5 years or older than 18 years of age; presence of diabetes mellitus, overweight and obesity, hypertension, dyslipidemia, autoimmune disease, acute or chronic infection, chronic liver, kidney or lung disease, growth failure, and immunodeficiency; tobacco smoke exposure; antihypertensive or antihyperlipidemic drug medication; family history of coronary artery disease or dyslipidemia. All volunteers with eligible criteria who agreed to participate in the study were included until the target sample size is reached (Figure 1).

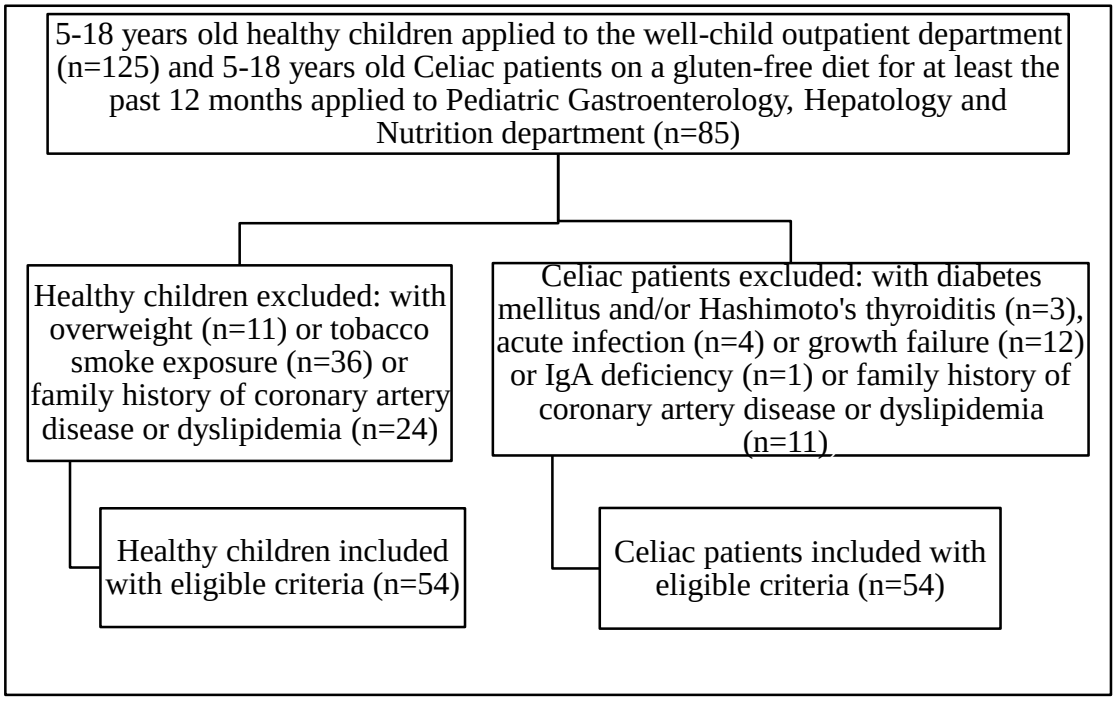

Figure 1. Flow chart of the study population selection

Weight, stature, upper arm and chest circumferences, and triceps skinfold thickness measurements were collected as anthropometric data. Body mass index (BMI) value was calculated (kg/m^2). Blood pressure measurements were made by mercury sphygmomanometer following at least 10 minutes rest and in sitting position. Anthropometric and blood pressure measurements were performed by the same pediatrician (Ö.T.). Serum lipid profile [triglyceride, total cholesterol and high density lipoprotein (HDL), low density lipoprotein (LDL), very low density lipoprotein (VLDL), and cholesterol], glucose, C-reaktive protein, sedimentation, 25(OH) Vitamin D, transaminases (AST, ALT), calcium, phosphor, alkaline phosphatase (ALP), albumine, vitamin B12, folate, ferritine levels and complete blood count values in venous blood samples drawed following at least 12 hours night fasting were obtained. Vitamin D levels between 20-100ng/mL were accepted as sufficient, levels between 12-20ng/mL were accepted as insufficient, and levels less than 12ng/mL were accepted as deficiency[14]. tTg-IgA levels were analysed in an accredited laboratory with enzyme-linked immunosorbent assay (Duzen Laboratory Group, Euroimmun analyzer) and higher than 20U/mL of tTg-IgA levels were accepted as positive. Vertebra bone mineral density (BMD) measurements scanned by dual X-ray absorptiometry in local radiology laboratory within the previous six months were noted by researching patient files. A BMD z-score of less than -2 defined as osteopenia[15]. According to

patients' self-reports, following gluten-free diet was classified as strict, not fully compliant, and noncompliant.

Carotid intima-media thickness and epicardial adipose tissue thickness measurements

The echocardiographic examination was performed via Vivid E9 Pro Ultrasound System (GE Medical Systems, Canada) using 3 and 6 MHz transducers by the same pediatric cardiologist (D.K.) to measure cIMT and EATT. Each measurement was performed at rest and in supine position without sedation to confirm normal anatomy and function. The cIMT was measured using the 11L-D probe. The carotid artery images were obtained by inverting the head 45 degrees to the contralateral side while the children were lying on the supine position. Measurements were taken following intima-media thickness proximal to the carotid bulb 1 cm and three measurements were taken for each patient, and the averages were used for analysis. EATT was measured on the free wall of the right ventricle, perpendicular to the wall, and from the parasternal long axis view at end-diastole for three cardiac cycles.

Statistical analysis

Statistical Package for the Social Sciences (SPSS) version 21.0 (IBM SPSS Corp.; Armonk, NY, USA) was used for statistical analysis. Shapiro-Wilk test and histograms were used to test for normality. For continuous variables mean±standard deviation and median (min-max) values, for categorical variables numbers (n) and percentages (%) were given as descriptive statistics. Independent groups were compared with Student t-test or Mann Whitney U test. Groups by type of following gluten-free diet were compared with ANOVA. Relationships between continuous variables were examined with Pearson's correlation coefficient and related variables analysed with regression analysis, and estimating equations were equated. Categorical variables were compared with Chi-square test. Whether vitamin D level of <20 ng/mL was a risk factor in the patient group was examined with logistic regression analysis and Odds ratio, 95% confidence interval were given. Statistical significance level was set as $p < 0.05$.

RESULTS

The patient and control groups did not differ in age and gender ($p>0.05$). The mean age was 11.9±3.8 years and mean disease duration was 4.1±2.1 years in the patient group. Histopathological classifications were Marsh type IIIa in three, Marsh type IIIb in five, Marsh type IIIc in 46 patients. There was tTg-IgA positivity in 32 (59.3%) patients. Following gluten-free diet was declarated as strict in 34 (63%), not fully compliant in 17 (31.5%), and noncompliant in 3 (5.5%) of the patients. Frequency of tTg-IgA positivity was not different between patients following strict, not fully compliant, and noncompliant gluten-free diet (71%, 50%, and 66.7%, respectively; $p=0.364$). Mean triceps skinfold thickness was significantly lower in the patient group than the control group (10.9±4.4 mm vs. 13.9±5.3 mm; $p=0.003$) while other anthropometric and blood pressure measurements were not statistically different (Table 1).

Except vitamin D levels, biochemical results were statistically similar and in normal range in both patient and control groups (Table 1). Median value of vitamin D was sufficient (22.5 ng/mL) in the control group while it indicated vitamin D insufficiency (19 ng/mL) in the patient group ($p=0.002$). The risk of vitamin D level of not being sufficient was 2.68 times higher in the patient group (95% CI=1.19-6.03; $p=0.017$).

The tTg-IgA positive and negative patient groups did not differ according to age, gender, anthropometric values, and frequency of following strict gluten-free diet. Except HDL cholesterol, other lipid levels were significantly lower in tTg-IgA positive group than tTg-IgA negative group ($p<0.05$). Comparison of tTg-IgA positive and negative groups is seen in Table 2.

Means of cIMT and EATT were significantly higher in the patient group than the control group (0.50±0.07 mm vs. 0.45±0.04 mm and 5.68±0.90 mm vs. 4.22±0.76 mm, respectively; $p<0.0001$) (Table 1). Means of cIMT and EATT were not different between tTg-IgA positive and negative patient groups ($p=0.746$, $p=0.176$, respectively) (Table 2).

Means of cIMT and EATT in both tTg IgA positive and negative patients were significantly higher than healthy children's values (for cIMT $p= 0.0004$, $p<0.0001$ and for EATT $p=0.019$, $p<0.0001$, respectively) (Table 3).

Means of cIMT were 0.50±0.08 mm, 0.51±0.06 mm, and 0.47±0.02 mm in patients following strict, not fully compliant, and noncompliant gluten-free diet,

respectively. Means of EATT were 5.76±0.92 mm, 5.54±0.97 mm, and 5.63±0.17 mm in patients following strict, not fully compliant, and noncompliant gluten-free diet, respectively. The patient groups by type of following gluten-free diet did not differ in cIMT and EATT (p=0.747, p=0.722, respectively).

Means of cIMT were 0.49±0.05 mm and 0.52±0.10 mm in patients with vitamin D level of <20 ng/mL and ≥20 ng/mL, respectively (p=0.217). Means of EATT were 5.61±0.94 mm and 5.78±0.87 mm in patients with vitamin D level of <20 ng/mL and ≥20 ng/Ml, respectively (p=0.519).

While EATT and cIMT were weakly correlated in the control group (r=0.387, p=0.005), there was no correlation in the patient group (r=0.245, p=0.086). tTg-IgA, vitamin D, BMD and BMD Z-score values were not significantly correlated with cIMT and EATT in the patient group (for cIMT: p=132, p=0.596, p=0.704, p=0.503 and for EATT: p=0.300, p=0.295, p=0.694, p=0.324, respectively). In the patient group, variables correlated with cIMT and EATT were BMI, systolic and diastolic blood pressures (for cIMT: r=0.310, p=0.029; r=0.294, p=0.038; r=0.283, p=0.046 and for EATT: r=0.515, p<0.0001; r=0.415, p=0.003; r=345, p=0.014, respectively).

Table 1. Demographic, anthropometric, biochemical, and echocardiographic characteristics in the patient and healthy control groups.

	Celiac patients (n=54)	Healthy controls (n=54)	p
Age (years)	11.9±3.8	11.3±3.4	0.395*
Gender (Female) (%)	51.8	48.1	0.180‡
Weight (kg)	36.3±14.3	41.4±14.1	0.077*
Height (cm)	140.7±20.8	147.6±18.1	0.801*
BMI (kg/m^2)	17.4±3.0	18.4±3.1	0.114*
Upper arm circumference (cm)	20.2±3.6	21.4±3.2	0.077*
Chest circumference (cm)	70 (49-86)	71 (52-83)	0.180†
Skinfold thickness (mm)	10.9±4.4	13.9±5.3	0.003*
Systolic BP (mm Hg)	100 (85-120)	100 (80-100)	0.631†
Diastolic BP (mm Hg)	60 (40-90)	70 (40-90)	0.306†
Glucose (mg/dl)	97.0±20.5	94.3±10.4	0.399†
Triglycerides (mg/dl)	71.5 (27-178)	92.5 (34-203)	0.062†
Total cholesterol (mg/dl)	144.6±30.2	150.8±22.6	0.246*
HDL cholesterol (mg/dl)	54.2±11.9	49.8±12.6	0.073*
LDL cholesterol (mg/dl)	72.1±21.0	79.8±22.0	0.777*
VLDL cholesterol (mg/dl)	14.1 (5.4-35.0)	18.2 (6.8-40.0)	0.104†
25(OH) vitamin D (ng/ml)	19 (4-40)	22.5 (9-50)	0.002†
Calcium (mg/dl)	9.8±0.4	9.8±0.3	0.267*
Phosphorus (mg/dl)	4.6 (3.1-6.3)	4.9 (3.6-5.7)	0.219†
ALP (IU/L)	188.1±76.3	209.9±76.7	0.157*
AST (IU/L)	23 (14-55)	20 (10-57)	0.113†
ALT (IU/L)	13 (7-30)	12.5 (5-34)	0.132†
Albumine (g/dl)	4.6 (4.0-5.1)	4.7 (4.2-5.1)	0.443†
CRP (mg/L)	0.8 (0.1-7)	0.7 (0-7.5)	0.904†
Sedimentation (mm/h)	4.5 (2-17)	4 (2-20)	0.433†
Hemoglobin (g/dl)	12.7 (8.1-15.8)	12.7 (8.5-16.7)	0.581†
Ferritin (ng/ml)	27.3±20.2	34.3±17.2	0.065*
Vitamin B12 (pg/ml)	271 (140-878)	323 (169-802)	0.461†
Folate (ng(ml)	10.2 (3-20)	8.9 (4-20)	0.112†
cIMT (mm)	0.50±0.07	0.45±0.04	<0.0001*
EATT (mm)	5.68±0.90	4.22±0.76	<0.0001*

*Student t test, data are mean±SD; †Mann Whitney U test, data are median (min-max); ‡Chi-square test, data are percentages.
BMI: body mass index; BP: blood pressure; HDL: high density lipoprotein; LDL: low density lipoprotein; VLDL:very low density lipoprotein; ALP:alkalane phosphatase, AST:aspartate transaminase; ALT:alanine aminotransferase; CRP: C reactive protein; cIMT:carotid intima media thickness; EATT:epicardial adipose tissue thickness.

Table 2. Comparison of tTg-IgA positive and negative Celiac patients

	tTg-IgA negative Celiac patients (n=22)	tTg-IgA positive Celiac patients (n=32)	p
Age (years)	11.4±4.0	12.3±3.7	0.424*
Gender (Female) (%)	50	53.1	0.835‡
Strict GFD declaration (%)	54.5	68.7	0.376‡
BMI (kg/m²)	17.1±2.7	17.6±3.3	0.560*
Skinfold thickness (mm)	11.1±3.7	10.8±4.8	0.852*
Systolic BP (mm Hg)	100 (90-120)	100 (85-120)	0.589†
Diastolic BP (mm Hg)	60 (50-90)	60 (40-80)	0.683†
Glucose (mg/dl)	99.3±11.9	95.2±9.9	0.407*
Triglycerides (mg/dl)	92 (40-178)	65 (27-154)	0.017†
Total cholesterol (mg/dl)	156.8±30.2	135.7±27.4	0.013*
HDL cholesterol (mg/dl)	55.1±10.4	53.6±13.0	0.669*
LDL cholesterol (mg/dl)	79.1±23.2	66.9±17.9	0.041*
VLDL cholesterol (mg/dl)	18.4 (8-35)	13 (5.4-30)	0.016†
25(OH) vitamin D (ng/ml)	19 (5.3-40)	17.7 (4-32)	0.701†
CRP (mg/L)	0.7 (0.1-7)	1 (0.1-4.3)	0.693†
Sedimentation (mm/h)	5 (2-13)	4 (2-17)	0.960†
Hemoglobine (g/dl)	13.0±1.2	12.5±1.3	0.201*
Ferritine (ng/ml)	30.6±13.5	25.5±12.2	0.396*
BMD (g/cm²)	156.7±33.5	141.0±29.4	0.087*
BMD z-score	-0.75±1.31	-1.40±1.1	0.065*
Osteopenia (%)	9.1	21.9	0.171‡
tTg-IgA antibody (U/L)	11.4 (5-20)	66 (20.4-300)	<0.0001*
cIMT (mm)	0.50±0.09	0.51±0.07	0.746*
EATT (mm)	5.48±0.89	5.84±0.91	0.176*

*Student t test, data are mean±SD; †Mann Whitney U test, data are median (min-max); ‡Chi-square test, data are percentages.
GFD:gluten free diet; BMI:body mass index; BP:blood pressure; HDL:high density lipoprotein; LDL:low density lipoprotein; VLDL:very low density lipoprotein; CRP: C reactive protein; BMD:bone mineral density; tTg-IgA:tissue transglutaminase immunglobuline A antibody; cIMT:carotid intima media thickness; EATT:epicardial adipose tissue thickness.

Table 3. Comparison of echocardiographic measurements between healthy controls and tTg-IgA positive and negative Celiac patients

	tTg-IgA negative Celiac patients (n=22)	Healthy controls (n=54)	p	tTg-IgA positive Celiac patients (n=32)	Healthy controls (n=54)	p
cIMT (mm)	0.50±0.09	0.45±0.04	0.019*	0.51±0.07	0.45±0.04	0.0004*
EATT (mm)	5.48±0.89	4.22±0.76	<0.0001*	5.84±0.91	4.22±0.76	<0.0001*

*Student t test, data are mean±SD
tTg-IgA:tissue transglutaminase immunglobuline A antibody; cIMT:carotid intima media thickness; EATT:epicardial adipose tissue thickness.

DISCUSSION

The present study is the first research to assess preclinical atherosclerosis by evaluating cIMT and EATT concurrently in pediatric CD cases. At the end of the mean disease duration of 4.1 years, the cIMT and EATT of children with CD were found to be higher than those of healthy peers, regardless of tTg-IgA status. Also, the findings demonstrated that children with CD had an increased risk for vitamin D level of not being sufficient which is known to be a risk factor for atherosclerosis. Literature data on the cardiovascular complications in pediatric patients with CD are limited. Systemic inflammation and diffuse immunological activation in CD are considered to be mechanisms that explain the development of atherosclerosis[16,17].

The atherosclerotic process has a long asymptomatic phase and in this subclinical phase, atherosclerosis

can be detected using non-invasive methods like cIMT and EATT measurements with echocardiography[18,19]. Increased cIMT is indicative of early hemodynamic changes or early arterial damage with no clinical signs. It is associated with coronary risk factors and increased risk of cardiovascular events[18]. It has been determined that epicardial adipose tissue contains a higher rate of inflammatory mediators in patients with coronary heart disease compared to healthy individuals[19]. The effect of EATT on the development of coronary heart disease has been explained by the absence of a physical barrier between epicardial adipose tissue and coronary artery walls and myocardium. Increased EATT has been associated with coronary heart disease, total atherosclerotic plaque burden, cIMT, and levels of traditional risk factors[20]. Hence, cIMT and/or EATT have been evaluated as early markers of atherosclerosis in pediatric cases with several diseases, including CD[10,21-25].

In the present study, cIMT and EATT were not statistically different between tTg-IgA positive and negative patient groups, and there was no correlation between tTg-IgA level and echocardiographic measurements. Also, Demir et al. detected no significant difference between such groups[10]. However, they reported that total cholesterol level was lower in children with CD compared to healthy children, tTg-IgA level was correlated with cIMT, and cIMT was lower in children who strictly adhered to the gluten-free diet than in healthy children. Based on these findings, the authors concluded that a gluten-free diet might be anti-atherogenic[10]. In the present study, the skinfold thickness of the patients was thinner, and the triglyceride and total cholesterol levels were lower in patients with tTg-IgA positivity. These findings were acceptable for a disease presenting with intestinal mucosal damage and malabsorption as main manifestations. tTg-IgA negativity occurs six to twelve months after starting a gluten-free diet[26].

Therefore, in tTg-IgA negative patients, the tTg-IgA status during the period older than 12 months might have affected the atherosclerotic profiles. In young adults with CD, De Marchi et al. measured cIMT to be significantly higher than the healthy group of the same age and found a significant decrease in cIMT after six to eight months of gluten-free diet compared to the baseline values[12]. Thus, we suggest that cIMT and its relationships with tTg-IgA level and gluten-free diet should be monitored with serial evaluations.

In previous studies, EATT was presented as a facilitating marker for grading cardiovascular disease risk, assessing subclinical target organ damage, and evaluating cardiovascular disease progression in children and adolescents[21-23]. EATT was found to be increased in children with a family history of type 2 diabetes mellitus but without clinical or biochemical diabetic signs[21]. EATT and cIMT were also higher in obese adolescents with a metabolic syndrome than healthy individuals[22]. In a study conducted with children with Familial Mediterranean Fever, increased EATT was reported and EATT was found to be correlated with onset age and duration of the disease. The authors suggested that EATT measurement could be used as a marker for the evaluation of atherosclerosis risk in people of all ages[23]. To the best of our knowledge, among the studies investigating the atherosclerosis risk profile of children with CD, this is the first study evaluating EATT. Our findings showed that children with CD had higher EATT than healthy peers. We suggest that EATT and its relationship with CD activity can be monitored with serial evaluations to screen atherosclerosis risk.

A correlation between cIMT and EATT independent of other coronary heart disease risk factors has been reported[27]. We considered that the reason for not detecting a similar correlation in the patient group but detecting in the control group in the current study was the relatively smaller size of the sample. Further studies with larger sample size should entirely investigate atherosclerotic risk factors and atherosclerotic early markers and define cut-off values of cIMT and EATT for subclinical/clinical atherosclerosis in children with CD.

We found a high correlation between EATT and BMI as similarly shown in a previous study[24]. Baroncini et al. measured cIMT in hypertensive children and adolescents to evaluate vascular damage and early atherosclerosis and found that cIMT was significantly increased in the hypertensive group regardless of age, sex, and BMI[25]. We also found positive correlations between cIMT, EATT and both systolic and diastolic blood pressures. Thus, evaluating cIMT, EATT and traditional risk factors for cardiovascular disease together may provide a rational approach to manage atherosclerosis.

An important finding of this study was that there was an increased risk for vitamin D insufficiency in pediatric CD cases. Muscogiuri et al. emphasized that vitamin D deficiency was a risk factor for

cardiovascular disease, atherosclerotic plaque development in peripheral arteries, and vessel wall calcification[28]. The authors stated that at advanced ages when the anti-inflammatory and antioxidant effect of vitamin D diminishes and the need for its effect on mineral metabolism increases, the deficiency of this vitamin could be the basis for the development of atheroma[28]. However, we did not find a significant relationship between 25 (OH) Vitamin D level and cIMT or EATT. This finding could be explained by the patient population consisting of children, not adults.

The effect of gluten-free diet on cardiovascular disease risk factors has not been clearly known. One of the two studies published in 2013 reported the anti-atherosclerotic effect of gluten-free diet and the other demonstrated its atherogenic effect[29,30]. Due to the malabsorpion, reduced cholesterogenesis, increased biliary secretion, or high fecal elimination, CD patients may have lower level of total cholesterol[31,32]. Also, in the present study, tTG-IgA positive patients had lower level of total cholesterol. Although we found that total cholesterol and triglyceride levels in tTG-IgA positive patients were significantly lower than those of tTG-IgA negative patients, we cannot certainly comment on the effect of gluten-free diet on the cardiovascular profile because of the similarity of the cIMT and EATT measures and lipid profile of within normal range in tTG-IgA negative and positive patients. Previous studies suggested that treatment with a gluten-free diet led to the improvement of the imbalanced lipid profile of CD patients[33,34]. In addition to this, based on our findings, we suggest that treatment with a gluten-free diet may lead to the improvement of the imbalanced vitamin D and bone mineral, and ultimately atherosclerotic profiles. Yet already, BMD has been reported to be inversely and independently associated with structural and functional measures of atherosclerosis[35].

Evaluating cIMT and EATT concurrently is the main strength of this study. As far as we know, this is the first time that a comparison of cIMT and EATT between cases with CD and healthy peers has been conducted in children. However, there are several limitations of this study. First, only self-reports on following gluten-free diet were included without an objective measure of components of diet. The compliance to gluten-free diet was verified using only tTG-IgA, deamidated gliadin peptide antibodies were not used. Secondly, physical activity was not considered while sedantary behavior was reported to predispose children with CD to atherosclerosis[32]. Thirdly, this was a cross-sectional survey, so we can not determine a direct etiologic relationship.

In conlsuion we demonstrated that children with Celiac Disease had higher cIMT and EATT than healthy peers, regardless of the adherence to gluten-free diet and tTG-IgA positivity. cIMT and/or EATT measurements by echocardiography present as an easy and reliable method to investigate subclinical atherosclerosis in children with celiac disease.

Yazar Katkıları: Çalışma konsepti/Tasarımı: DK, ÖT, YU; Veri toplama: DK, ÖT; Veri analizi ve yorumlama: DK, ÖT, MTŞ; Yazı taslağı: DK, ÖT; İçeriğin eleştirel incelenmesi: YU; Son onay ve sorumluluk: DK, ÖT, MTŞ, YU; Teknik ve malzeme desteği: -; Süpervizyon: YU; Fon sağlama (mevcut ise): yok.
Etik Onay: Bu çalışma için Mersin Üniversitesi Klinik Araştırmalar Etik Kurulundan 21.09.2017 tarih ve 256 sayılı kararı ile etik onay alınmıştır.
Hakem Değerlendirmesi: Dış bağımsız.
Çıkar Çatışması: Yazarlar çıkar çatışması beyan etmemişlerdir.
Finansal Destek: Yazarlar finansal destek beyan etmemişlerdir.

Author Contributions: Concept/Design : DK, ÖT, YU; Data acquisition: DK, ÖT; Data analysis and interpretation: DK, ÖT, MTŞ; Drafting manuscript: DK, ÖT; Critical revision of manuscript: YU; Final approval and accountability: DK, ÖT, MTŞ, YU; Technical or material support: -; Supervision: YU; Securing funding (if available): n/a.
Ethical Approval: Ethical approval was obtained for this study from the Ethical Committee of Clinical Research of Mersin University with the decision dated 21.09.2017 and numbered 256.
Peer-review: Externally peer-reviewed.
Conflict of Interest: Authors declared no conflict of interest.
Financial Disclosure: Authors declared no financial support

REFERENCES

1. Shannahan S, Leffler DA. Diagnosis and updates in celiac disease. Gastrointest Endosc Clin N Am. 2017;27:79-92.
2. Kelly CP, Bai JC, Liu E, Leffler DA. Advances in diagnosis and management of celiac disease. Gastroenterology. 2015;148:1175-86.
3. Tye-Din JA, Galipeau HJ, Agardh D. Celiac disease: a review of current concepts in pathogenesis, prevention, and novel therapies. Front Pediatr. 2018;6:350.
4. Emilsson L, Lebwohl B, Sundström J, Ludvigsson JF. Cardiovascular disease in patients with coeliac disease: a systematic review and meta-analysis. Dig Liver Dis. 2015;47:847-52.
5. Kerola AM, Rollefstad S, Semb AG. Atherosclerotic cardiovascular disease in rheumatoid arthritis: impact of inflammation and antirheumatic treatment. Eur Cardiol. 2021;16:e18.
6. Naaraayan A, Nimkar A, Jesmajian S, Gitler B, Acharya P. Atherosclerotic cardiovascular disease prevalence among patients with celiac disease in the united states: an observational study. Mayo Clin Proc. 2021;96:666-76.

7. Jamnik J, Jenkins DJ, El-Sohemy A. Biomarkers of cardiometabolic health and nutritional status in individuals with positive celiac disease serology. Nutr Health. 2018;24:37-45.
8. van den Oord SC, Sijbrands EJ, ten Kate GL, van Klaveren D, van Domburg RT, van der Steen AF et al. Carotid intima media thickness for cardiovascular risk assessment: systematic review and meta-analysis. Atherosclerosis. 2013;228:1-11.
9. Villasante Fricke AC, Iacobellis G. Epicardial adipose tissue: clinical biomarker of cardio-metabolic risk. Int J Mol Sci. 2019;20:5989.
10. Demir AM, Kuloğlu Z, Yaman A, Fitöz S, Nergizoglu G, Kansu A. Carotid intima-media thickness and arterial stifness as early markers of atherosclerosis in pediatric celiac disease. Turk J Pediatr. 2016;58:172-9.
11. Raitakari O, Pahkala K, Magnussen CG. Prevention of atherosclerosis from childhood. Nat Rev Cardiol. 2022;19:543-54.
12. De Marchi S, Chiarioni G, Prior M, Arosio E. Young adults with coeliac disease may be at increased risk of early atherosclerosis. Aliment Pharmacol Ther. 2013;38:162-9.
13. Gujral N, Freeman HJ, Thomson AB. Celiac disease: prevalence, diagnosis, pathogenesis and treatment. World J Gastroenterol. 2012;18:6036-59.
14. Munns CF, Shaw N, Kiely M, Specker BL, Thacher TD, Ozono K et al. Global consensus recommendations on prevention and management of nutritional rickets. J Clin Endocrinol Metab. 2016;101:394-415.
15. Estrada A, Ramnitz MS, Gafni RI. Bone densitometry in children and adolescents. Curr Opin Obstet Gynecol. 2014;26:339-46.
16. Korkmaz H, Sozen M, Kebapcilar L. Increased arterial stiffness and its relationship with inflammation, insulin, and insulin resistance in celiac disease. Eur J Gastroenterol Hepatol. 2015;27:1193-9.
17. Santoro L, De Matteis G, Fuorlo M, Giupponi B, Martone AM, Landi F et al. Atherosclerosis and cardiovascular involvement in celiac disease: the role of autoimmunity and inflammation. Eur Rev Med Pharmacol Sci. 2017;21:5437-44.
18. Rafati M, Rahimzadeh MR, Moladoust H. Evaluation of atherosclerosis severity based on carotid artery intima-media thickness changes: a new diagnostic criterion. Ultrasound Med Biol. 2019;45:2950-7.
19. Shimabukuro M, Hirata Y, Tabata M, Dagvasumberel M, Sato H, Kurobe H et al. Epicardial adipose tissue volume and adipocytokine imbalance are strongly linked to human coronary atherosclerosis. Arterioscler Thromb Vasc Biol. 2013;33:1077–184.
20. Kim S, Chung J, Kwon B, Song S, Choi W. The associations of epicardial adipose tissue with coronary artery disease and coronary atherosclerosis. Int Heart J. 2014;55:197–203.
21. Mahfouz RA, Alzaiat A, Yousry A. Relationship of epicardial fat thickness with endothelial and cardiac functions in children with family history of type 2 diabetes mellitus. Echocardiography. 2015;32:28-33.
22. Elshorbagy HH, Fouda ER, Kamal NM, Bassiouny MM, Fathi WM. Evaluation of epicardial fat and carotid intima-media thickness in obese children. Iran J Pediatr. 2016;26:e2968.
23. Uluca U, Demir F, Ece A, Şen V, Güneş A, Aktar F et al. Assessment of epicardial adipose tissue thickness and the mean platelet volume in children with familial Mediterranean fever. Ital J Pediatr. 2015;41:15.
24. Eren E, Koca B, Ture M, Guzel B. Epicardial adiposity in children with obesity and metabolic syndrome. Iran J Pediatr. 2014;24:411-7.
25. Baroncini LAV, Sylvestre LC, Baroncini CV, Pecoits RF. Assessment of carotid intima-media thickness as an early marker of vascular damage in hypertensive children. Arq Bras Cardiol. 2017;108:452-7.
26. De Leo L, Aeschlimann D, Hadjivassiliou M, Aeschlimann P, Salce N, Vatta S et al. Anti-transglutaminase 6 antibody development in children with celiac disease correlates with duration of gluten exposure. J Pediatr Gastroenterol Nutr. 2018;66:64-8.
27. Boyraz M, Pirgon O, Akyol B, Dundar B, Cekmez F, Eren N. Importance of epicardial adipose tissue thickness measurement in obese adolescents, its relationship with carotid intima-media thickness, and echocardiographic findings. Eur Rev Med Pharmacol Sci. 2013;17:3309-17.
28. Muscogiuri G, Annweiler C, Duval G, Karras S, Tirabassi G, Salvio G et al. Vitamin D and cardiovascular disease: From atherosclerosis to myocardial infarction and stroke. Int J Cardiol. 2017;230:577-84.
29. Zanini B, Mazzoncini E, Lanzarotto F, Ricci C, Cesana BM, Villanacci V et al. Impact of gluten-free diet on cardiovascular risk factors. A retrospective analysis in a large cohort of coeliac patients. Dig Liver Dis. 2013;45:810-5.
30. Martin J, Geisel T, Maresch C, Krieger K, Stein J. Inadequate nutrient intake in patients with coeliac disease: results from a German dietary survey. Digestion. 2013;87:240-6.
31. Gajulapalli RD, Pattanshetty DJ. Risk of coronary artery disease in celiac disease population. Saudi J Gastroenterol. 2017;23:253-8.
32. Rybak A, Wierzbicka A, Socha P, Stolarczyk A, Cukrowska B, Obrycki Ł et al. Atherosclerotic risk factors in children with celiac disease. Gastroenterol Res Pract. 2020:6138243.
33. Forchielli ML, Fernicola P, Diani L, Scrivo B, Salfi NC, Pessina AC et al. Gluten-free diet and lipid profile in children with celiac disease: comparison with general population standards. J Pediatr Gastroenterol Nutr. 2015;61:224-9.
34. Soares FL, de Oliveira Matoso R, Teixeira LG, Menezes Z, Pereira SS, Alves AC et al. Gluten-free diet reduces adiposity, inflammation and insulin resistance associated with the induction of PPAR-

alpha and PPAR-gamma expression. J Nutr Biochem. 2013;24:1105-11.
35. Hyder JA, Allison MA, Barrett-Connor E, Detrano R, Wong ND, Sirlin C et al. Bone mineral density and atherosclerosis: the Multi-Ethnic Study of Atherosclerosis, Abdominal Aortic Calcium Study. Atherosclerosis. 2010;209:283-9.