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Evaluation of epicardial adipose tissue and carotid intima-media thickness as a marker of atherosclerosis in patients with inflammatory bowel disease

Nergiz Ekmen,1 Güray Can,2 Ahmet Yozgat,3 Hatice Can,4 Muhammed Fatih Bayraktar,5 Muhammed Emin Demirkol,6 Meral Akdogan Kayhan,7 Hadi Sasani 8
1 Department of Gastroenterology. Gazi University. Faculty of Medicine. Ankara. Turkey
2 Department of Gastroenterology. Abant Izzet Baysal University. Faculty of Medicine. Bolu. Turkey
3 Department of Gastroenterology. Ufuk University. Faculty of Medicine. Ankara. Turkey
4 Department of Nephrology. İnönü University. Faculty of Medicine. Malatya. Turkey
5 Department of Cardiology. Abant Izzet Baysal University. Faculty of Medicine. Bolu. Turkey
6 Department of Internal Medicine. Abant Izzet Baysal University. Faculty of Medicine. Bolu. Turkey
7 Department of Gastroenterology. Bilkent State Hospital. Ankara. Turkey
8 Department of Radiology. Namik Kemal University. Faculty of Medicine. Tekirdag. Turkey.

Corresponding Author: Nergiz Ekmen M.D.
Gazi University, Faculty of Medicine, Department of Gastroenterology, 60150, Yenimahalle, Ankara, Turkey.
Phone: +90 505 677 05 57  Fax: +90 312 221 32 02
E-mail: dr_nergisekmen@hotmail.com

ABSTRACT

Background and study’s purpose
It was aimed to compare carotid intima media (CIMT) and epicardial adipose tissue (EAT) measurements, which are considered as markers in detecting early atherosclerosis, in healthy control and inflammatory bowel diseases (IBD).

Methods
In a total of 60 IBD patients (25 Crohn disease and 35 ulcerative colitis) and 60 healthy patients as control group, were included in the study. The measurement of CIMT and EAT were performed by using echocardiography and ultrasonography, respectively. Statistical
analysis was employed for the relationship between the parameters.

**Main results**
The thickness of bilateral (right and left) CIMT and EAT were found to be significantly higher in IBD than those of the control group (P <0.05). There was a positive correlation between EAT and bilateral (right and left) CIMT in IBD patients (p<0,05).

**Conclusion**
IBD is associated with increased thickness of EAT and CIMT. Because chronic inflammation in IBD may increase the risk of atherosclerotic heart disease, only measuring the thickness of EAT and CIMT can be used as an objective, easy, simple, affordable, non-invasive and accessible assessment method in order to screen this risk.

**Keywords**
Carotid intima-media thickness, Echocardiography, Atherosclerosis, Pericardium, Adipose tissue, Inflammatory bowel diseases.

**Abbreviations**
IBD, inflammatory bowel disease; EAT, the epicardial adipose tissue; CD, Crohn disease; UC, ulcerative colitis ; R-CIMT, the thickness of right carotid intima media; L-CIMT, the thickness of left carotid intima media;

**INTRODUCTION**
Coronary artery disease and myocardial infarction are the major causes of morbidity and mortality worldwide (1). In addition to the factors such as smoking, cholesterol levels, body mass index (BMI), frequency of exercising and blood pressure, which are shown to play a role in cardiovascular diseases, chronic inflammation is also shown to contribute to the development of atherosclerosis (2). Carotid intima media thickness (CIMT) has been proved to be associated with major cardiovascular risk factors and is a precious guide to the atherosclerotic events (3). Epicardial adipose tissue (EAT), a visceral adipose tissue compartment, is located between the pericardium and myocardial layers and in direct contact with the coronary vessels and myocardium without any tissue and fascia (4). Like other white adipose tissues, EAT has an
endocrine function capable of secreting the hormones and inflammatory cytokines (4, 5). The secretion and expression of pro-inflammatory cytokines such as interleukin-6 (IL-6), interleukin-1β (IL-1β), and tumour necrosis factor-α (TNF-α) are higher at EAT than subcutaneous adipose tissue in the patients with coronary artery disease, and pathologically enlarged EAT is significantly correlated with increased cardiovascular disease risk (6). Thanks to the widespread availability of non-invasive imaging methods such as echocardiography, computed tomography and magnetic resonance imaging, EAT measurement which is a reliable cardiovascular risk predictor, is applied more increasingly (7). Echocardiography is a simple, inexpensive and easily available measurement tool in healthcare facilities. Inflammatory bowel disease (IBD) including two systemic diseases as ulcerative colitis (UC) and Crohn's disease (CD), result inflammation in the intestine (8). In addition to the bowel, one-third of IBD patients experience extra-intestinal symptoms in eyes, skin, musculoskeletal system, and cardiovascular system (9). Systemic or local inflammatory burden differs depending on the factors such as the duration of disease, prevalence of bowel involvement and disease activity in the patients. Recently, remarkable data has been put forward in recent years, suggesting that systemic inflammation plays a role in the development of atherosclerosis in IBD patients (10). This data brings to mind the thought of investigating the development of atherosclerosis among the patients with systemic inflammatory disease. However, there are a limited number of studies conducted on IBD, which progresses with inflammation for many years, and also there is no consensus on results of these studies, either (11-15).

This study aims to scrutinize the relationship between CIMT and EAT thickness by using sonographic methods (echocardiography, carotid Doppler ultrasonography) echocardiography and the significance of both in diagnosing early atherosclerosis in IBD patients.

MATERIAL AND METHODS

Ethical Issue

The study was a cross-sectional single center study, approved by the Clinical Research Ethics Committee of the institution (No: 116, Decision number: 2019/51, Dated: 07.03.2019). Informed consents of all patients were obtained.

Patient Selection
Patients admitted to the gastroenterology outpatient clinic during a 4-month period between April and July 2019 were evaluated in terms of inclusion and exclusion criteria. Inclusion criteria: Patients over age 18 years and those who were in remission, were included in the study. Exclusion criteria: Patients with diabetes mellitus, hypertension, hyperlipidaemia, acute and chronic kidney disease, thromboembolic disease, chronic lung disease, malignancy, autoimmune vasculitis and other autoimmune diseases, cardiac disease; those using drugs (anti-TNF, active steroids) and patients with active disease in terms of IBD were excluded from the study. The control group was specified by matching the age and gender from volunteers without any known chronic or cardiac disease and not using heart-acting drugs.

The diagnosis of IBD was made clinically, endoscopically, radiologically and histologically according to ECCO consensus criteria. Partial Mayo score was used for ulcerative colitis patients and Harvey-Bradshaw Index was used for Crohn's patients to assess disease activation. The personal and clinical information, medical history and IBD-related clinical data of the patients were obtained from hospital data system.

**Laboratory parameters**

Pre-prandial blood glucose, C reactive protein (CRP) and erythrocyte sedimentation rate (ESR) were measured by standard procedures from blood samples taken after fasting overnight. While patients who had no disease related to lipid profile in their previous examinations were included in the study, no additional blood tests were performed for a new lipid profile at the time of study. In both groups, systolic (SBP) and diastolic (DBP) blood pressure were measured three times on the right arm in seated position after ten-minute resting, then a mean value was figured out. BMI was calculated by dividing the square of the height by weight.

**CIMT and EAT measurement methods**

EAT thickness was measured by echocardiography with the 4 MHz probe (Vivid 9 Pro, GE Vingmed, Milwaukee, Wisconsin, USA) in the left lateral decubitus position, from the right ventricular free wall on the parasternal long axis images. CIMT were determined by carotid Doppler ultrasonography, using a 12 MHz superficial probe of the same device, which provides an image from linear angle (figure 1). All echocardiographic measurements were made in the IBD and control group according to the standards recommended by the
American Echocardiography Association (16).

**Statistical analysis**

The conformity of continuous variables to normal distribution was evaluated by using visual (histogram and probability graphs) and analytical methods (Kolmogorov-Smirnov/Shapiro-Wilk tests). Independent sample t test was used for the comparative analysis between the two groups in the data conforming to the normal distribution as a result of the normality analyses, and the Mann-Whitney U test was used for non-conforming data. The chi-square ($\chi^2$) test was used in the comparison analysis made for categorical variables between the independent groups, and spearman correlation analysis was used for the correlation analysis between two factors. P values (2-sided) below 0.05 were considered statistically significant. All analyses were conducted using SPSS version 22. Comparative data are shown as Mean ± Standard error.

**RESULTS**

Of 95 IBD patients who applied to the gastroenterology outpatient clinic within a 4-month period, 35 patients (with one or more exclusion criteria) were excluded from the study. Thus, in a total of 60 IBD patients (25 CD and 35 UC) were enrolled in the study. The average age and gender distribution of UC (35 patients), CD (25 patients) and healthy control group (60 volunteers) were similar ($p=0.387$; $p=0.070$, respectively). Among the patients included in the study all patients with UC were receiving 5-aminosalicylates and all patients with CD were receiving azathioprine treatment. Twenty patients (57.1%) with ulcerative colitis and ten patients (40%) with Crohn's disease had previously one or more steroid usage history.

There was no difference between IBD and control group in terms of smoking and BMI ($p>0.05$). There was no significant difference between IBD and control group in terms of other cardiovascular parameters (Table 1). The median duration of disease in IBD was found to be 4 years (1-20). ESR, CRP, DBP and RCIMT, LCMIT and EAT (figure 2A, 2B, 2C) measurements were significantly higher in IBD compared to the control group ($p<0.05$). Comparisons between clinical and laboratory parameters between groups are summarized in Table 1.

A moderate negative correlation between EAT thickness and R-CIMT was found in the control group ($r = -0.308$, $p = 0.017$); while in IBD patients, there was a moderate positive correlation between EAT and R-CIMT ($r = 0.474$, $p <0.001$) and a weak positive correlation.
with L-CIMT (r = 0.275, r = 0.035) (Figure 3A, 3B). No correlation was found between age and EAT, R-CIMT, L-CIMT in the control group (Table 2). In IBD patients R-CIMT, L-CIMT and EAT measurements showed a moderately positive correlation with age (r=0.566, p<0.001; r=0.472, p<0.001; r=0.553, p<0.001, respectively).

No correlation was detected between the disease duration and EAT, R-CIMT, L-CIMT in IBD (Table 2). No statistically significant association was found between glucose, gender and smoking with CIMT and EPA in the healthy control and IBD group (p>0.05).

**DISCUSSION**

IBD is thought to play a role in the development of atherosclerosis due to its chronic inflammatory nature in recent years (15, 17). Although traditional cardiovascular risk factors are not higher in IBD patients compared to the general population, there are higher rates of cardiovascular mortality and morbidity and cardiovascular events in IBD patients (18-20). This high risk may be associated with inflammation-mediated atherosclerosis (18). It is known that inflammation may have an independent role in the pathogenesis of atherosclerosis or act synergistically with traditional risk factors (21). CIMT is a widely accepted inflammatory marker in clinical practice in predicting early atherosclerosis (22).

EAT and CIMT thickness, a valuable predictor of atherosclerotic vascular disease, are considered as an independent risk factor for coronary artery disease (23, 24). CIMT and EAT have been studied together in some chronic inflammatory diseases in recent years and have been shown as a new parameter in predicting atherosclerosis (25, 26). To the best of our knowledge, there are quite limited number of studies evaluating the thickness of EAT and CIMT together to predict early atherosclerosis in IBD patients. In our study, CIMT thickness was found to be significantly higher in IBD patients compared to the control group, suggesting that they have increased the risk of early atherosclerosis. The results in the current study were similar to the several studies investigating CIMT and early atherosclerosis in IBD (15, 27-29). In a study involving only CD patients (12) and in three different studies including CD and UC patients, there was no difference in CIMT thickness between IBD patients and the control (11, 13, 14). It was shown that the risk of ischemic heart disease decreased in IBD patients using anti-inflammatory 5-ASA treatment compared to the group not using it and the fact that immunomodulatory therapy (such as azathioprine) reduces arterial stiffness (20) , it is also noteworthy that the CIMT thickness is higher in the IBD group compared to the healthy control group.
In the current study, while EAT showed positively correlation with CIMT and the age in IBD group, there was a moderate negative correlation between EAT and CIMT in the healthy control group and none showed correlation with the age. EAT was significantly higher in IBD patients than in the control group. The negative correlation between EAT and CIMT in the healthy control group can be explained by the absence of chronic inflammatory processes in this group. As the reason for the increase in CIMT and EAT with age in the IBD group, it can be thought that the inflammatory load created by IBD with advancing age may have accelerated the atherosclerotic process.

In a study conducted by Uysal et al. in IBD patients, CIMT thickness was found to be similar with the controls while EAT thickness was found higher in IBD patients than in the controls. Also, in the same study it was found that CIMT and EAT thickness correlated only in CD patients (15).

In a study conducted with ankylosing spondylitis patients, a correlation was found between the disease duration and EAT and CMT (25), while in another study with patients with psoriasis, there was no correlation between the disease duration and EAT (26). The reason may be due to the nature of the disease which has activation and remissions periods. As the frequency and length of activation are different in each patient, the effect of inflammation in the body differs from person to person in the same group of patients. In our study, there was no correlation between disease duration and EAT and CIMT.

There were several limitations in our study. The first limiting factor was that all IBD patients were in remission, therefore, the effect of being in the active period in IBD could not be evaluate on EAT and CIMT. The second limiting factor was the exclusion of an IBD group receiving anti-TNF therapy. There are recent studies reporting the conclusion that biologics such as anti-TNF and immunomodulatory therapy such as methotrexate cause cardiovascular improvement in rheumatoid arthritis patients with chronic inflammation. We think that the inclusion of a group receiving anti-TNF therapy would be meaningful in terms of investigating the effects of these drugs on CIMT and EAT in IBD patients. The third limiting factor is the inability to examine the effects of active steroid use in the study population with a history of steroid use, considering the cardio protective effect of corticosteroid treatment used in IBD patients (20). Another limitation was partly small number of the study population in the current study.

In conclusion, the usefulness of EAT thickness and CIMT, which can be evaluated as non-invasive, easily applicable method and markers of atherosclerosis and cardiovascular
disease, increases in patients with IBD. The data obtained from the study showed that both EAT thickness and CIMT can be considered to be used more effectively to diagnose early atherosclerosis in patients with IBD. We think large-group multicentre prospective studies are needed to evaluate the effect of EAT and CIMT measurement on early atherosclerosis and cardiovascular mortality and morbidity in IBD patients.

AUTHORSHIP
All authors were involved in the study concept and design, analysis and interpretation of the data, and the drafting and critical revision of the manuscript for important intellectual content. Nergiz Ekmen edited and reviewed the manuscript, and is the article guarantor. All authors read and approved the final manuscript.

FUNDING
The authors declared that this study has received no financial support.

AVAILABILITY OF DATA AND MATERIALS
Patient’s data was obtained from hospital data system.

CONFLICT OF INTEREST
No conflict of interest was declared by the authors.

INFORMED CONSENT
Written informed consent was obtained from the patient who participated in this study.

ETHICAL APPROVAL
(No: 116, Decision number: 2019/51, Dated: 07.03.2019)

HUMAN RIGHTS
Written informed consent was obtained from the patients who participated in this study.

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21. Han QF, Wu L, Li T, Meng XY, Yao HC. There is a link between carotid intima media thickness and coronary artery disease: It might be inflammation. Int J Cardiol. 2016;203:1144-5.


Table 1. Comparison of the clinical and laboratory characteristics of study participants.

<table>
<thead>
<tr>
<th>Variables</th>
<th>IBD(n=60)</th>
<th>Control(n=60)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>36.90±11.53</td>
<td>36.61±8.09</td>
<td>0.877a</td>
</tr>
<tr>
<td>Gender: Male</td>
<td>35(58.3)</td>
<td>34(56.7)</td>
<td>0.853b</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>25(41.7)</td>
<td></td>
</tr>
<tr>
<td>Smoker, n (%)</td>
<td>19(31.7)</td>
<td>21(35)</td>
<td>0.699b</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>24.35±4.84</td>
<td>25.97±4.30</td>
<td>0.059c</td>
</tr>
<tr>
<td>Disease duration (years)</td>
<td>4 (1-20)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ESR, mm/h</td>
<td>11.50(1.00-32.00)</td>
<td>5.50(1.00-15.00)</td>
<td>&lt;0.001c</td>
</tr>
<tr>
<td>CRP, mg/L</td>
<td>1.00(0.10-16.00)</td>
<td>0.58(0.06-8.00)</td>
<td>0.046c</td>
</tr>
<tr>
<td>FPG, mg/dL</td>
<td>93.09±9.73</td>
<td>89.93±8.92</td>
<td>0.073a</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>128.53±9.43</td>
<td>127.61±10.74</td>
<td>0.623a</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>79.03±7.84</td>
<td>74.80±4.91</td>
<td>0.001a</td>
</tr>
<tr>
<td>EF, %</td>
<td>60(55-66)</td>
<td>62(55-69)</td>
<td>0.258c</td>
</tr>
<tr>
<td>LV-ESD, cm</td>
<td>2.8(2.4-3.2)</td>
<td>2.8(2.1-3.4)</td>
<td>0.398c</td>
</tr>
<tr>
<td>LV-EDD, cm</td>
<td>4.6(4.1-5.2)</td>
<td>4.7(3.4-5.3)</td>
<td>0.300c</td>
</tr>
<tr>
<td>AAR, cm</td>
<td>3.2(2.8-3.9)</td>
<td>3.2(2.9-3.7)</td>
<td>0.383c</td>
</tr>
<tr>
<td>AR, cm</td>
<td>3.2(0.8-4.2)</td>
<td>3.2(2.8-3.7)</td>
<td>0.858c</td>
</tr>
<tr>
<td>PW, cm</td>
<td>0.9(0.7-2.9)</td>
<td>0.8(0.7-1)</td>
<td>0.085c</td>
</tr>
<tr>
<td>IVS, cm</td>
<td>0.9(0.7-4.5)</td>
<td>0.9(0.7-1)</td>
<td>0.098c</td>
</tr>
<tr>
<td>LA, cm</td>
<td>3.4(2.8-4)</td>
<td>3.2(2.8-4)</td>
<td>0.006c</td>
</tr>
<tr>
<td>RCIMT, mm</td>
<td>0.50(0.40-0.70)</td>
<td>0.40(0.40-0.50)</td>
<td>&lt;0.001c</td>
</tr>
</tbody>
</table>
Table 2. Correlation of study parameters with EAT thickness, CIMT and age.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control</th>
<th></th>
<th></th>
<th>IBD</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EAT</td>
<td>r</td>
<td>p</td>
<td>EAT</td>
<td>r</td>
</tr>
<tr>
<td>RCIMT</td>
<td>-0.308</td>
<td>0.017</td>
<td></td>
<td>0.474</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LCIMT</td>
<td>-0.087</td>
<td>0.507</td>
<td></td>
<td>0.275</td>
<td>0.035</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EAT</td>
<td>-0.163</td>
<td>0.213</td>
<td></td>
<td>0.553</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>RCIMT</td>
<td>0.071</td>
<td>0.592</td>
<td></td>
<td>0.566</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LCIMT</td>
<td>-0.152</td>
<td>0.245</td>
<td></td>
<td>0.472</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Disease duration, years</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EAT</td>
<td></td>
<td>0.179</td>
<td>0.171</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RCIMT</td>
<td></td>
<td>0.206</td>
<td>0.114</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LCIMT</td>
<td></td>
<td>0.202</td>
<td>0.126</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

BMI indicates body mass index; IBD, inlamatuary bowel disease. RCIMT and LCIMT, right and left carotid intima-media thickness; EAT, epicardial adipose tissue thickness; FPG, fasting plasma glucose, ESR, erythrocyte sedimentation rate; CRP, C-reactive protein, SBP = systolic blood pressure and DBP = diastolic blood pressure. a: The independent-samples test, b: chi-square analysis c: MannWhitney U test, EF; ejection fraction, , LV-ESD; left ventricular end-systolic diameter, LV-EDD; left ventricular end-diastolic diameter AAR; Assendan aortic root diameter, AR; aortic root diameter, PW; Posterior Wall thickness, IVS; interventricular septum thickness, LA; left atrium diameter.
Figure 1. Illustration shows CIMT measurements. While the patient was lying in a supine position, the patient was moved manually by giving the neck an angle of approximately 20 degree towards the opposite side of the neck, with the probe positioned parallel to the common carotid artery (CCA). Measurements were taken from three points: A) the first 2 cm proximal part of the internal carotid arteries, B) bilateral CCA, C) 2 cm proximal to carotid bifurcation. CIMT measurements were performed with longitudinal examination using B-Mode imaging across the distance defined as the clearance of vascular lumen echogenicity and media/adventitia echogenicity.

Figure 2A. Comparison of epicardial adipose tissue thickness in control and IBD.
Figure 2B. Comparison of right carotid intima media thickness in control and IBD.

Figure 2C. Comparison of left carotid intima media thickness in control and IBD.

Figure 3A. Correlation between epicardial adipose tissue and right carotid intima-media thickness.

Figure 3B. Correlation between epicardial adipose tissue and left carotid intima-media thickness.