# Evaluation of the possible involvement of Ad-36-induced adipogenesis and coronary artery disease development in mediastinal adipose tissue samples

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## SUMMARY

Mediastinal fat has been suggested to be associated with cardiovascular diseases such as carotid stiffness, atherosclerosis and coronary artery calcification. We investigated the possible role of Ad-36-induced obesity in the pathogenesis of the coronary artery disease (CAD). Ad-36 DNA was investigated in the anterior mediastinal fat tissue samples of obese adults with CAD. Seventy-five obese adults with left main coronary artery (LMCA) disease, 28 non-obese adults with valvular heart diseases, and 48 healthy individuals without cardiovascular problems were included as the obese patient group (OPG), non-obese patient group (NOG) and healthy control group (HCG), respectively. We also simultaneously investigated Ad-36 antibodies by serum neutralization test (SNA), and measured leptin and adinopectin levels. Ad-36 antibodies were detected only in 10 patients (13.3%) within the 75 OPG. A statistically significant difference was detected between OPG, NOG and HCG in terms of Ad-36 antibody positivity ($p<0.05$). Ad-36 DNA was not detected in mediastinal tissue samples of OPG and NOP without PCR inhibitors. We suggest that Ad-36 may not have an affinity for mediastinal adipose tissue in obese patients with left main CAD and valvular heart diseases. Ad-36 antibody positivity results are not sufficient to reach a causal relationship.

*Keywords*: Adenovirus-36, obesity, mediastinal fat, left main coronary artery disease.

## INTRODUCTION

Obesity has been regarded an independent risk factor for cardiovascular diseases, and a relation between obesity and high blood pressure, serum cholesterol, and low levels of high-density lipoprotein-cholesterol (HDL-C) has been reported in the literature [1]. Fat is mainly accumulated as subcutaneous fat, but can it also be observed in the abdomen, thorax (as epicardial, mediastinal and/or intramyocardial fat), the pancreas and in skeletal muscles [2].

Fat stored in the intrathoracic space is known as "extra-pericardial adipose tissue" and "mediasti-

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**Table 1** - Characteristics of obese and non-obese patient groups, and healthy control group.

| | *Obese patients' group** | *Non-obese patients' group* | *Healthy control group* |
|---|---|---|---|
| Number | 75 | 28 | 48 |
| Gender (female, male) | F=37, M=38 | F=15, M=13 | F=25, M=23 |
| Age (mean ± sd) | 58.64±9.28 | 57.18±10.44 | 56.54±9.78 |
| BMI (kg/m$^2$) (range/median) | 30, 44/33 | 20, 29/23 | 20, 25/23 |
| WC (cm, mean ± sd) | 112.4±5.3 | 88.1±4.7 | 84.4±5.3 |
| WHR (mean ± sd) | 1.08±0.03 | 0.76±0.05 | 0.77±0.07 |

*Abbrevations:* F: Female; M: Male; WC: waist circumference; WHR: waist to hip ratio.
**Criteria of obesity:* BMI ≥30 kg/m$^2$ (Body mass index).

nal fat," but fat stored around the myocardium is called "epicardial adipose fat" and "intramyocardial fat." While mediastinal fat is the fat existing on the outer surface of the heart, epicardial fat is located within the cardiac sac (pericardium) [3].

Epicardial and mediastinal adipose tissue are linked to several cardiovascular diseases. In some studies, the volume of epicardial adipose tissue has been suggested to be associated with the progression of coronary artery calcification, myocardial infarction, atrial fibrillation, carotid stiffness, and atherosclerosis [3-7]. On the other hand, mediastinal fat has also been suggested to be associated with carotid stiffness, atherosclerosis and coronary artery calcification [8, 9].

Human adenovirus-36 (Ad-36) is the first human virus linked with obesity and Ad-36-induced adiposity in a chicken model, as shown by Dhurandhar et al. [10]. In several studies, hyperplasia and hypertrophy in the adipocytes of mice, rats, and monkeys were demonstrated after Ad-36 infection [10-13].

Here, we investigate the possible role of Ad-36-induced obesity in the pathogenesis of the coronary artery disease (CAD). For this purpose, we investigated Ad-36 DNA in the anterior mediastinal fat tissue samples of obese adults with CAD. We also simultaneously investigated Ad-36 antibodies by serum neutralization test (SNA), and we measured leptin and adinopectin levels using the enzyme-linked immunosorbent assay (ELISA) in the same patients.

## MATERIALS AND METHODS

*Patients and control groups*

This cross-sectional study was conducted between April 2015 and December 2016 in the Istanbul Mehmet Akif Ersoy Thoracic and Cardiovascular Surgery Training and Research Hospital, Department of Cardiovascular Surgery Ethical approval was obtained for the mediastinal adipose tissue samples. Seventy-five obese (body mass index; BMI ≥30 kg/m$^2$) adults with left main coronary artery (LMCA) disease, 28 non-obese (BMI ≤25 kg/m$^2$) adults with valvular heart diseases, and 48 healthy individuals (BMI ≤25 kg/m$^2$) without cardiovascular problems were included as the obese patient group (OPG), non-obese patient group (NOG) and healthy control group (HCG), respectively. Patients with acute infectious disease, cancer, chronic liver or renal disease, or individuals who were seropositive for hepatitis B and C were excluded from this study. All of the groups were matched in terms of age and gender with recorded demographic data ($p>0.05$) (Table 1).

*Collection of adipose tissue and serum specimens*

Three samples of 50 mg mediastinal fat tissue samples were collected from the anterior mediastinum, located on the external surface of the pericardium of OPG and NOG, during routine cardiovascular surgical procedures. The samples were weighed and stored in Allprotect™ Tissue Reagent (Life Technologies, Foster City, CA). The samples were incubated in the reagent at 2-8°C for one night and then transferred to -20°C until the study. We also collected blood samples from the patients in all of the groups (OSG, NOG and HCG). All of the samples were stored at -80°C until the study. Total cholesterol, total triglyceride, HDL, and LDL levels of the groups were measured using routine biochemical methods (Siemens Advia 2400, Chemistry System, Germany).

*Virus titration and serum neutralization assay for viral antibodies*

We used the SNA that is considered to be the golden standard for detecting specific neutralizing antibodies against Ad-36. The SNA was performed using the "constant virus-decreasing virus" method. A commercially obtained Ad-36 ATCC-VR 1610 (Rockville, MD, USA) strain was grown on an A549 cell line (human lung carcinoma cell line, ATCC-CCL-185). Since the infectious titer of the virus is accepted as the dilution with 50% positivity of the virus in live cells, the infectious virus titer was calculated according to the Spearman-Kärber method [14]. This test was performed in accordance with the test procedure defined by Dhurandar et al. [15]. The serum samples were thawed from -80°C at room temperature and the test sera were heat-inactivated in a water bath at 56°C for 1 h and serially diluted (1:4 to 1:512) in 96-well plates. The titrated virus suspension was immediately thawed at 37°C in a water bath. One hundred TCID-50 of the Ad-36 working stock was added to serum samples in a ratio of 1:1 and incubated for 1 h at 37°C. One hundred µl of the mixture was added to each of the wells containing confluent A549 cells ($2x10^4$ cells per well). Positive (virus and cell but no serum) and negative (only cell) serum controls were prepared for each plate. The plates were incubated at 37°C for 11 days, and the presence of cytopathic effect (CPE) was recorded. Serum samples without CPE in dilutions of 1:8 or higher were considered to be positive for the presence of neutralizing antibodies to the human Ad-36. The presence of CPE in virus control wells was also assessed.

*Serum leptin and adiponectin levels with ELISA*

For the analysis of the serum leptin (Human Leptin ELISA DRG Diagnostics, Germany, Catalog No: EIA2395) and adiponectin (Adiponectin, Assaypro LLC, USA, Catalog No: EA2500-1) were evaluated using commercial ELISA kits. The kits were used according to the manufacturer's guidelines.

*DNA extraction and PCR*

Mediastinal adipose tissue samples were minced using scalpels under sterile conditions. Genomic DNA was purified from 50 mg of minced tissue using a commercial DNA purification kit (High Pure PCR Template Preparation Kit, Roche Diagnostics GmbH, Mannheim, Germany) according to the manufacturer's instructions and stored at -80°C until further use.

The samples were screened for Ad-36 DNA with polymerase chain reaction (PCR) using primer sets (primer set I for single step in-house PCR and primer set II for nested-PCR) of the *E4 orf1* gene region of Ad-36 described previously [13,16]. For the PCR assay (single step in-house PCR and first round of nested PCR), a 10 µl DNA template was added to the 40 µl reaction mixture consisting of 1 µl forward primer (50 pmol/µl) and 1 µl reverse primer (50 pmol/µl), 5 µl 10X Taq buffer with KCI, 3 µl 25 mM $MgCl_2$, 1 µl dNTP stock (200 mM each dATP, dTTP, dCTP and dGTP) (Fermentas®, Lithuania), 1.25 U Taq DNA polymerase (Fermentas®, Lithuania) and 28.75 µl nuclease-free water. Amplification was carried out on a thermal cycler (PTC-100, MJ Research Inc., USA) under the following conditions: after initial denaturation for 2 min at 95°C, 35 cycles of 1 min at 94°C, 1 min at 58°C and 2 min at 72°C. The final extention was performed at 72°C for 5 min. Three microliters of the reaction product were used for nested PCR (under the same conditons as those described previously). The amplification products were visualized on 1.5% agarose gel electrophoresis under ultraviolet light. The expected size of single step in-house PCR and nested PCR products were ~138 bp and ~650 bp, respectively. In the PCR assay, Ad-36 (ATCC-VR-1610) was used as a positive control. For the exclusion of the PCR inhibitors, all of the samples were tested in a separate run using beta globin primers.

*Statistical analysis*

We used Fischer's exact probability test to compare the frequency and percentages of the patient and control groups, and we used the Mann-Whitney U test and criteria for a normal distribution for comparing the two groups. Logistic regression tests for multivariate analyses were performed according to the conductive model. The biostatistical analysis of the study results was performed using the SPSS 21.0 (SPSS Inc., Chicago, IL, USA) program licensed to Istanbul University. The significance value was considered to be $p<0.05$.

## RESULTS

The waist-to-hip ratio (WHR) and waist circumferences (WC) indicate visceral adiposity

if WHR≥0.90 (0.85) cm for males (females) and WC>94 (80) cm for males (females). The mean WC was found to be 112.4±5.3, 88.1±4.7 and 84.4±5.3 cm for OPG, NOG and HCG, respectively. The mean WHR was found to be 1.08±0.03, 0.76±0.05 and 0.77±0.07 for OPG, NOG and HCG, respectively (Table 1).

Ad-36 antibodies were detected in 10 patients (13.3%) within the 75-patient OPG using SNA but not in other groups. A statistically significant difference was detected between OPG and NOG and HCG in terms of Ad-36 antibody positivity ($p<0.05$) (Table 2). Mean BMI, leptin, LDL, total triglyceride and total cholesterol levels were found to be higher in OPG patients, and adiponectin and HDL levels were found to be lower in OPG patients. Significant differences were noted between OPG patients and those in other groups in terms of mean BMI, leptin, adiponectin, HDL, LDL and total triglycerides ($p<0.05$). No significant differences were found between the groups in terms of total cholesterol.

Mean BMI was found to be higher in the Ad-36 antibody positive OPG patients, and adiponectin and leptin levels were found to be lower in the Ad-36 antibody positive OPG patients. Although no statistically significant differences were found in terms of serum total cholesterol, total triglyceride, leptin, adiponectin, HDL, LDL levels and BMI between the two groups ($p>0.05$) (Table 3), lower levels of mean total cholesterol and total triglyceride levels were found in the Ad-36 antibody-positive OPG patients. The OR was determined to be 11.54 ($p>0.05$) between the OPG group and other groups.

Ad-36 DNA was not detected in mediastinal tissue samples of OPG and NOP without PCR inhibitors. In the PCR, the ATCC-VR-1610 strain was

**Table 2** - Test results of obese and non-obese patient groups and healthy control group.

| *Parameters* | *Obese patients' group* | *Non-obese patients' group* | *Healthy control group* | *Statistical value* |
|---|---|---|---|---|
| BMI (kg/m$^2$) (mean ± sd)? | 34.43±3.63 | 23.28±2.025 | 22.70±1.45 | p<0.05 |
| HDL (mmol/L) (mean ± sd)? | 37.60±11.13 | 44.93±15.52 | 40.85±16.63 | p<0.05 |
| LDL (mmol/L) (mean ± sd) | 126.5±51.07 | 103.07±25.25 | 90.42±26.38 | p<0.05 |
| Total cholesterol (mmol/L) (mean ± sd) | 196.5±63.69 | 169.9±40.55 | 165.5±36.07 | p>0.05 |
| Total triglyceride (mmol/L) (mean ± sd) | 201.9±136.8 | 96.14±32.03 | 96.75±30.95 | p<0.05 |
| Leptin (ng/ml) (mean ± sd) | 14.63±9.54 | 3.02±2.33 | 4.27±4.05 | p<0.05 |
| Adiponectin (µg/ml) (mean ± sd) | 7.59±4.19 | 15.76±13.06 | 20.00±12.16 | p<0.05 |
| Ad-36 Ab (positive/negative) | 10/75 | 0/28 | 0/48 | p<0.05 |

*Abbrevations:* Ad-36 Ab: Adenovirus-36 Antibody, mean ± sd (standard deviation).
*Criteria of obesity:* BMI ≥30 kg/m$^2$.

**Table 3** - Comparison of BMI, HDL, LDL, leptin, adiponectin, cholesterol and triglyceride levels according to the presence of Ad-36 antibodies in the patients'group.

| | *Presence of Ad-36 Ab* | | *Statistical value* |
|---|---|---|---|
| | *Ad-36 Ab (+) (n=10)* | *Ad-36 Ab (-) (n=65)* | |
| BMI (n:75)<br>HDL (n:75)<br>LDL (n:75) | 34.71±4.01<br>35.60±8.52<br>105.4±48.57 | 34.38±3.60<br>37.90±11.50<br>129.8±51.03 | p>0.05<br>p>0.05<br>p>0.05 |
| Leptin (n:75) | 12.61±9.3 | 14.94±10.32 | p>0.05 |
| Adiponectin (n:75) | 7.11±3.38 | 7.67±4.32 | p>0.05 |
| Total cholesterol (n:75) | 163.1±49.46 | 201.6±64.38 | p>0.05 |
| Total triglyceride (n:75) | 139.1±49.37 | 211.6±143.5 | p>0.05 |

*Abbrevations:* BMI: Body mass index; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; Ad-36 Ab: Adenovirus-36 Antibody.

**Table 4** - Logistic regressions of variables.

| *Variables in the equation* | | | | | | |
|---|---|---|---|---|---|---|
| | *B* | *S.E.* | *Wald* | *df* | *Sig.* | *Exp (B)* |
| Ad-36 seropositivity | 5.518 | 7.968 | 0.480 | 1 | 0.196 | 210.12 |
| Gender | -2.607 | 1.101 | 5.611 | 1 | 0.018 | 0.075 |
| HDL | -0.070 | 0.031 | 5.074 | 1 | 0.024 | 0.932 |
| LDL | 0.028 | 0.013 | 4.472 | 1 | 0.034 | 1.028 |
| Total Triglyceride | 0.017 | 0.007 | 5.563 | 1 | 0.017 | 1.017 |
| Total Cholesterol | -0.010 | 0.010 | 0.896 | 1 | 0.271 | 0.990 |
| Leptin | 0.457 | 0.131 | 12.143 | 1 | 0.000 | 1.579 |
| Adiponectin | -0.369 | 0.096 | 14.638 | 1 | 0.000 | 0.692 |
| Constant | -0.628 | 1.257 | 0.250 | 1 | 0.617 | 0.534 |

*Abbreviations:* B: beta regression coefficient; S.E.: standard error; Wald: test statistics used for the determination of the meaning of variables, d.f.: degrees of freedom; Exp (B): exponent.

used as a positive control. In this study, gender, HDL, LDL, total triglyceride, leptin, adiponectin levels were determined to be risk factors in the multivariate analysis according to a logistic regression test of parameters such as demographic data, biochemical characteristics and the presence of Ad-36 seropositivity (p<0.005) (Table 4).

## DISCUSSION

Cardiovascular disease is one of the leading causes of death worldwide. Coronary artery disease is responsible for the high mortality rates, and significant LMCA disease among patients with CAD is associated with increased cardiac mortality [17]. As subjects become more obese, the major increase is shown in the extra-pericardial site (mediastinal adipose tissue). Mediastinal adipose tissue constitutes the majority of fat around the heart [18].

Because of the proximity of the myocardium to the coronary arteries and the ability of epicardial fat to produce cytokines, such fat is suggested to be involved in the pathogenesis of cardiovascular diseases more than pericardial fat (mediastinal). Increased pericardial (mediastinal) fat has been strongly associated with metabolic syndrome [19]. However, there is no definite concensus on this subject. For example, Yun et al., suggested that epicardial adipose tissue is associated with the severity and risk factors of CAD and correlated with serum adiponectin levels [20]. As shown previously, epicardial adipose tissue is supplied by branches of the coronary arteries, but mediastinal adipose tissue is supplied by the branches of the internal mammary arteries. It has been suggested that epicardial adipose tissue is more closely associated with the development of atherosclerosis [20]. On the other hand, Sicari et al. suggested that pericardial fat is a better cardiometabolic risk marker than epicardial fat because most cardiac fat consists of pericardial fat (about 77%), and pericardial fat thickness estimated via echocardiography is well correlated with BMI, WC, visceral fat, subcutaneous fat, blood pressure, insulin sensitivity, triglycerides, cholesterol, glucose and coronary heart disease risk [21]. Based on this conflicting information related to the importance of epicardial and mediastinal adipose tissues for CAD and also given ethical concerns, we decided to use mediastinal adipose tissue for Ad-36 DNA detection.

There have been very few studies involving the tissue affinity of Ad-36. To better understand tissue affinity of Ad-36, Pasarica et al. experimentally infected rats with Ad-36 [22]. Four days later, the infected rats exhibited 23% greater epididymal fat pad weight than their normal weight. These authors also detected very quickly viral DNA in the liver, brain, and adipose tissue of the Ad-36-infected rats. They concluded that Ad-36 suppresses systemic immune response and spreads widely [16]. The same researchers also infected the primary human adipose-derived stem/stromal cells (hASC) with Ad-36. Pasarica et al. concluded that Ad-36 has the potential to induce adipogenesis in

hASC [22]. On the other hand, they did not infect human subjects with Ad-36 for ethical reasons. In an animal model (marmosets), Ad-36 DNA was detected in various tissues 6 months after the animals were experimentally infected with Ad-36 [13]. Ad-36 infection was initially detected in the deposition of fat within the abdomen and chest cavities of the infected animals. Salahian et al. reported a case report indicating the presence of Ad-36 DNA in adipose tissues of humans [23]. In this case report, the authors detected Ad-36 DNA in a subcutaneous fat biopsy specimen of a 62-year-old male patient with disseminated lipomatosis.

Here, we focus on exploring the presence of a direct or indirect link between Ad-36-induced obesity and cardiovascular disorders in humans. To the best of our knowledge, there has only been one study on this subject performed by Bil-Lula et al. [24]. These authors studied 25 obese/overweight subjects with cardiac disorders but did not detect Ad-36 DNA in the adipose tissue of their study group. They finally suggested that Ad-36 exhibits no tropism to mediastinal adipose tissue and that Ad-36 tropism may be different in animal and human tissues. Bil-Lula et al. additionally suggested that factors other than Ad-36 may be responsible from the increased accumulation of fat around the heart in the mediastinum [24]. However, Ponterio et al. detected Ad-36 DNA in four adipose biopsy samples (19%) of the adipose tissue of 21 adult overweight or obese patients [25]. They concluced that some individuals may carry Ad-36 in their visceral adipose tissues.

We studied the mediastinal adipose tissues of 75 obese adults with LMCA disease and 28 non-obese adults with valvular heart diseases. We did not find Ad-36, but we detected Ad-36 antibodies in 10 members of the OPG by SNA. The results of our study also suggested that Ad-36 shows no tropism to mediastinal adipose tissue, which is consistent with the results of Bil-Lula et al. [24]. However, there are no other studies with which to compare our findings.

Goossens et al. analyzed 509 serum samples for Ad-36 antibodies using SNA and the visceral adipose tissue of 31 severely obese surgical patients for Ad-36 DNA [26]. They found that an Ad-36 seroprevalence of 5.5% that increased with age. However, no Ad-36 DNA was detected by PCR in visceral adipose tissues, consistent with our study results. These authors concluded that Ad-36 has no direct effect on increased BMI or obesity in humans in Western Europe. However, Goossens et al. did not exclude causality between Ad-36 and obesity because some Ad-36 infection-induced mechanisms were initially reported as inflammatory responses of pre-adipocytes and remodelling of human adipocytes [26].

After these unsuccesful attempts to find Ad-36 DNA in human adipose tissues, Goossens pointed out a mismatch between the high seroprevalence of Ad-36 and lower Ad-36 DNA detection in clinical samples [27]. Dhurandhar explained this mismatch as Ad-36-infected animals generally exhibiting Ad-36 DNA in their various organs within 4 days of infection [28]. However, the viral Ad-36 DNA prevalence declines with time. As an example, 5 months after Ad-36 infection, all Ad-36 infected mice had neutralizing antibodies, but only 60-70% of them had Ad-36 DNA in their adipose tissue. Furthermore, acute Ad-36 infections of humans may be missed, and Ad-36-infected clinical samples will be consequently underrepresented [28]. Dhurandhar attempted to explain why the tissue distribution of Ad-36 in animals are not the same as observed in humans [28]. However, only a few reports exist in the literature indicating Ad-36 DNA in human adipose tissues. We agree with the comments of Dhurandhar because the presence of Ad-36 antibodies does not indicate an actual Ad-36 infection [28]. Obesity has been initially purposed as a state of low-grade chronic inflammation by several researchers [29, 30]. We have also suggested in our previous studies that obesity may be a result of low-grade chronic inflammation as an immunological cascade that initially started with Ad-36 infection [31, 32].

There are several implications of our study. We detected type 2 diabetes in four out of 10 obese patients with left main CAD. However, Almgren et al., suggested that Ad-36 infection may be less frequent in individuals with type 2 diabetes or impaired glycemic control [33]. They explained this phenomen as Ad-36 seropositivity being more common in individuals with normal glucose tolerance than in diabetics. Almgren et al. studied the presence of antibodies against Ad-36, but seropositivity does not imply an acute Ad-36 infection, as noted by Goossens and Dhurandhar [27, 28, 33]. On the other hand, even though the finding was not statistically significant, the detec-

tion of four Ad-36 seropositive patients with type 2 diabetes necessitates further investigation.

Another implication of our study was that the mean leptin levels of obese patients with Ad-36 infection were insignificantly slightly lower in obese patients with Ad-36 seropositivity than in obese patients without Ad-36 seropositivity. As noted earlier, Ad-36 inhibits leptin gene expression, and there may consequently be an increase in lipid accumulation leading to obesity. The mechanism resulting obesity related to this hypothesis was explained as an Ad-36 infection increasing food intake by decreasing norepinephrine levels and leptin [34]. Furthermore, the detection of paradoxically lower levels of total serum cholesterol and triglyceride levels in the obese group with positive Ad-36 antibodies compared with the obese group without Ad-36 was consistent with the findings of Dhurandhar et al., and Atkinson et al., [12, 13, 35]. Paradoxically, the virus also appears to lower cholesterol and triglycerides while promoting weight gain, raising intriguing questions as to whether some component of the virus might prove to be valuable in developing a treatment for diabetes or high cholesterol.

Another implication of our study was the detection of significantly higher leptin and lower adinopectin levels in our OPG patients independent of the presence of Ad-36 seropositivity. The mechanism underlying this inverse correlation between leptin and adinopectin levels was explained by López-Jaramillo et al. [36]. These authors studied patients with severe CAD and abdominal obesity and showed that these individuals exhibited decreased plasma adiponectin and increased leptin levels compared with controls. A leptin/adiponectin imbalance was associated with increased WC. Type 2 diabetes mellitus and cardiovascular diseases are associated with abdominal obesity on the basis of a leptin/adiponectin imbalance. However, adinopectin levels were insignificantly lower in our obese patient group with Ad-36 infection. Gender, mean HDL, LDL, total cholesterol, total triglyceride, leptin, adiponectin levels were determined to be risk factor - but not Ad-36 seropositivity - in our multivariate analysis according to logistic regression ($p<0.005$).

There are some limitations to this study. First, we were unable to use three techniques for quantifying these adipose tissues, namely Magnetic Resonance Imaging (MRI), Echocardiography and Computed Tomography (CT). We only used WC and WHR to predict to indicate indicate visceral (mediastinal) adiposity.

## CONCLUSIONS

We suggest that Ad-36 had no affinity for mediastinal adipose tissue in obese patients with left main CAD and valvular heart diseases. For the 10 obese patients with Ad-36 seropositivity plus CAD, we hypothesize that the immunological mechanisms that were triggered by Ad-36 infection may have been involved in the enlargement of the visceral adipose tissue. Consequently, Ad-36 infection may have been contributed indirectly to the formation of CAD by increasing the size of the mediastinal adipose tissue. However, there is a need for more comprehensive serial cases to determine whether there is an involvement of Ad-36-induced obesity in the pathogenesis of CAD. We also suggest that an increased accumulation of fat around the heart in the mediastinum may be related to factors other than Ad-36 replication, as noted by Bil-Lula et al. [24].

**Conflict of interests**

The authors have declared that no competing interests exist. This research was presented as a poster in the 17th International Congress on Infectious Diseases (ICID) Hyderabad, 2016.

**Acknowledgements**

This research was supported by Scientific Research Projects Coordination Unit of Istanbul University. Project number: 23874. The poster presentation was supported financially by Istanbul University Research Fund with the project BEK-2016-20167. We also thank Esad Bonabi (Aydin University, Turkey), Gulsel Ayaz (Istanbul University, Turkey) for their technical assistance related to the poster presentation in the 17th International Congress on Infectious Diseases (ICID) Hayderabad, 2016.

**Ethics**

This study has been approved by the Clinical Research Ethics Committee of Istanbul University Cerrahpasa Medical Faculty (Number: 8304589/604.01/02.173630, Date: 04 September 2014). All of the patients were provided with information about the study and they signed written consent forms.

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