Microbial Pathogenesis 80 (2015) 57–62

Contents lists available at ScienceDirect

Microbial Pathogenesis

journal homepage: www.elsevier.com/locate/micpath

The role of adenovirus 36 as a risk factor in obesity: The first clinical study made in the fatty tissues of adults in Turkey

Sevgi Ergin [a], Eda Altan [b], Ozgur Pilanci [c], Serhat Sirekbasan [a], Oguz Cortuk [c], Utku Cizmecigil [b], Idris Ersin [c], Huseyin Elbey [d], Harika Oyku Dinc [a], Zafer Habip [a], Nuri Turan [b], Atilla Arinci [d], Juergen A. Richt [e], Valere J. Goossens [f], Asiye Karakullukcu [a], Banu Tufan Kocak [g], Suat Saribas [a], Selcuk Koksal [h], Huseyin Yilmaz [b], Bekir Kocazeybek [a, *]

[a] Istanbul University, Cerrahpasa Medical Faculty, Department of Medical Microbiology, Istanbul, Turkey
[b] Istanbul University, Veterinary Faculty, Department of Virology, Avcilar, Istanbul, Turkey
[c] Bagcilar Training and Research Hospital, Department of Plastic Reconstructive and Esthetic Surgery, Istanbul, Turkey
[d] Istanbul University, Istanbul Medical Faculty, Department of Plastic Reconstructive and Esthetic Surgery, Istanbul, Turkey
[e] Department of Diagnostic Medicine and Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS 66506, USA
[f] Maastricht University, Medical Centre, Department of Medical Microbiology, Maastricht, The Netherlands
[g] Erenkoy Mental Health and Neurology Training and Research Hospital, Istanbul, Turkey
[h] Istanbul University, Cerrahpasa Medical Faculty, Public Health Department, Istanbul, Turkey

ARTICLE INFO

Article history:
Received 18 September 2014
Received in revised form
20 February 2015
Accepted 22 February 2015
Available online 24 February 2015

Keywords:
Obesity
Adipose tissue
Adenovirus-36
Serum neutralization assay
PCR

ABSTRACT

Obesity which developes due to multifactorial reasons, was associated recently with human Adenovirus-36 (Ad-36). The aim of this study was to investigate the prevalence of Ad-36 antibodies in obese adults and also to investigate the DNA of Ad-36 in their adipose tissue. In this cross-sectional and case-control based study, 49 obese adults, with BMI $\geq$30 kg/m^2, and 49 non-obese adults, with BMI $\leq$25 kg/m^2, applied for esthetic purposes and were included in this study as patient and control groups, respectively. Adipose tissue samples, obtained by the lipoaspiration method, were studied by single-step PCR and nested-PCR methods. Simultaneously, the presence of Ad-36 antibodies and serum leptin and adiponectin levels were assessed by serum neutralization assay (SNA) and ELISA, respectively. Serum samples which didn't cause a cytopathic effect at $\geq$1:8 were accepted as positive. Ad-36 antibody was detected in 6 (12.2%) of 49 patients by SNA and was statistically significant ($p < 0.05$). Ad-36 DNA was not detected in any of the adipose tissue samples of the patient or control groups. Mean BMI and leptin levels were higher in the Ad-36-positive group, while adiponectin levels were found to be lower in the Ad-36-positive group. Although no statistically significant difference was found in cholesterol and triglyceride levels between the two groups ($p > 0.05$), lower mean serum cholesterol and triglyceride levels were found in the Ad-36-positive patients. In conclusion, we couldn't detect Ad-36 DNA in adipose tissue; however, we detected significantly higher Ad-36 antibody levels in the obese group compared to the non-obese group, according to the both univariant and multivariant analyses, suggesting that Ad-36 may play a role in obesity. There is a need for new and extended serial, particularly cohort and human-based, studies in order to have a clear understanding of the Ad-36-obesity relationship.

1. Introduction

Obesity is a disease that occurs when the energy intake of foods exceeds energy expenditure, and excess energy accumulates in adipose tissue as fat in the body [1]. According to the World Health Organization (WHO), persons with a body mass index (BMI) $\geq$ 30 kg/m^2 are considered obese [2]. In recent years, obesity

* Corresponding author. Istanbul University, Cerrahpasa Medical Faculty, Department of Medical Microbiology, Cerrahpasa Street, 34098 Istanbul, Turkey.
E-mail address: bzeybek@istanbul.edu.tr (B. Kocazeybek).

http://dx.doi.org/10.1016/j.micpath.2015.02.008
0882-4010/

has been described as a significant health challenge worldwide, with associations to other disease states and a rapid increase in prevalence, it has been proposed that the development of obesity depends upon the multifactorial etiologies. The rapid spread of obesity suggests a potential role for pathogenic factors (e.g., bacteria and viruses) as potential etiological agents in the progression of obesity, and the term "infectobesity" has been suggested [3]. Within this context, avian adenovirus SMAM-1, identified as a capable agent causing obesity in animals, was also reported as the first virus to be associated with human obesity [4,5]. Ad-36 has since also been associated with human obesity [6–11].

Because experimental infection in human subjects cannot be performed for ethical reasons, the affinity of Ad-36 for adipose tissue has been experimentally demonstrated in animal models (e.g., chicken, mice and monkeys), while an association of Ad-36 with human obesity has also been reported [12–15]. By indicating a direct effect of Ad-36 on human adipocytes, adenoviruses were added to the potential causative agents list of obesity [16]. In many studies, a correlation between obesity and Ad-36 has been shown via serum neutralization assay (SNA), the accepted gold standard test for the detection of Ad-36 [16–19]. Recently, there has been a significant increase in the number of serological and molecular-based studies on adenovirus performed with children and adult samples [6–8,11,20–22].

Here we aimed to investigate the presence of the Ad-36 antibody, and its correlation with leptin and adinopectin levels, as well as the presence of Ad-36 DNA in adipose tissues of obese adults, thereby determining any potential role for Ad-36 role in obesity.

2. Materials and methods

2.1. Patients and control groups

This study was conducted as a cross-sectional and case-control based study. Forty-nine adults (29 female and 20 male, mean age 36.94 ± 12.89) diagnosed as obese with BMI ≥ 30 kg/m^2 and admitted to Plastic, Reconstructive and Esthetic Surgery Clinics of various hospitals between March 2013 and February 2014 were included as the patient group. Forty-nine non-obese adults with BMI ≤25 kg/m^2 (29 female and 20 male, mean age 33.6 ± 11.77) admitted to the same hospitals for esthetic purposes, were included as the control group in the same period. Patient and control groups were matched in terms of age and gender with recorded demographic data ($p > 0.05$) (Table 1).

The distributions of BMI were different for patient and control groups when obesity criteria of BMI ≥30 kg/m^2. BMI was higher in patient group than control group. It was shown as side-by-side box-whisker plots of BMI in patient and control groups (Fig. 1). This study was approved by the Clinical Research Ethics Committee of Istanbul University Cerrahpasa Medical Faculty (Number: B.30.2.İST.0.30.90.00/, Date: 03 April 2012). All patients were given information about the study and signed consent forms.

Table 1
Characteristics of patient group and control group.

Characteristic/Variable	Patient group	Control group
Number	49	49
Gender (female, male)	F = 29, M = 20	F = 29, M = 20
Age, mean ± sd	36.94 ± 12.89	33.61 ± 11.77
BMI, median (range), kg/m^2	30 (30–45)	25 (20–25)

Abbreviations: F: Female; M: Male; Criteria of obesity: BMI ≥30 kg/m^2; Mean ± sd: Mean ± standard deviation.

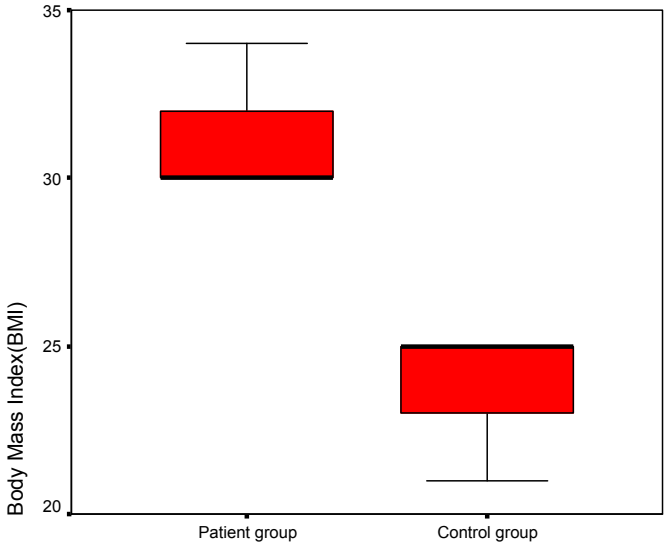

Fig. 1. Side-by-side box-whisker plots of BMI in patient and control groups when obesity criteria of BMI ≥30 kg/m^2.

2.2. Collection of adipose tissue and serum specimens

Adipose tissue samples (hip, thigh, and abdominal areas) were taken from obese and non-obese groups with lipoaspiration methods to investigate the presence of Ad-36 DNA. ELISA assay were used to investigate leptin and adiponectin levels, and SNA was used to investigate Ad-36 neutralizing antibody levels from serum samples of all cases obtained during routine blood collection prior to lipoaspiration. All samples were stored at −80 °C until use. Both serum cholesterol and triglyceride levels of the groups were measured during routine biochemical tests (Siemens Advia 2400, Chemistry System, Germany).

2.3. Immunological tests

2.3.1. Serum neutralization assay

The presence of antibodies directed against Ad-36 was investigated by SNA in blood samples taken during routine biochemical tests before lipoaspiration in the groups of obese and non-obese adults.

2.3.1.1. Virus titration. Human Ad-36 (Ad-36, ATCC-VR-1610) was obtained from the American Type Culture Collection ATCC, Rockville, MD, USA). The stock virus was grown using A549 cell (human lung carcinoma cell line, ATCC-CCL-185) cultures. The titer of the virus stocks was calculated according to cytopathic effect (CPE) in 50% of the wells containing A549 cells. TCID-50 of the virus was determined using serial 10-fold dilutions of the virus stock as described by Reed-Muench et al. [23].

2.3.1.2. Serum neutralization assay for viral antibodies. The assay was performed as described by Dhurandhar et al. [12]. Briefly, 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 at a ratio of 1:1 and incubated for 1 h at 37 °C. The mixture (100 μl) was added to each of the wells containing confluent A549 cells (2×10^4 cells per well). Each test serum was run in triplicate. Controls (cells alone; cells and virus; positive control serum and cells but no virus; and positive control serum and cells with virus)

were included in each run. Plates were incubated at 37 °C for 8–11 days and the presence of CPE was recorded. Serum samples without CPE in dilutions of 1:8 or higher were considered positive for the presence of neutralizing antibodies to the human Ad-36. Presence of CPE in virus control wells was also assessed.

2.3.2. Serum leptin and adiponectin levels with ELISA

Serum leptin (AssayMax Human Leptin ELISA kit, Assaypro, USA, Catalog No: EL2001-1) and adiponectin levels (AssayMax Human Adiponectin ELISA kit, Assaypro, USA, Catalog No: EA2500-1) were evaluated using commercial ELISA kits and sera of obese and non-obese adults. The study was performed according to the manufacturer's recommendations.

2.4. Molecular assay

2.4.1. DNA extraction and PCR

Adipose tissue samples were minced using surgical scalpels under sterile conditions. Genomic DNA was purified from 1 g of minced tissue using a commercial DNA purification kit (High Pure PCR Template Preparation Kit, Roche Diagnostics GmbH, Mannheim, Germany) according to the instructions of the manufacturer and were stored at −80 °C until 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) for the *E4 orf1* gene region of Ad-36 described previously [13,14]. Nucleotides of the primers are given in Table 2. In the PCR assays (single step in-house PCR and first round of nested-PCR), 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 10 × Taq buffer with KCl, 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 condition: 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. Final extension was performed at 72 °C for 5 min. Three microliters of the reaction product were used for nested-PCR (under the same conditions as those described previously). The amplification products were visualized on 1.5% agarose gel electrophoresis under UV-light. The expected size of single step in-house PCR and nested-PCR products were ~138 and ~650 bp, respectively. In the PCR assay, Ad-36 (ATCC-VR-1610) was used as a positive control. For the exclusion of PCR inhibitors, all samples were tested in a separate run using beta globin primers (primer set III).

Table 2
The primer sets used in this study.

	Direction	Sequence (5′–3′)	Product size
Primer set I			
Ad-36 E4 F	Sense	5'ggc ata cta acc cag tcc gat g 3′	~138 bp.
Ad-36 E4 R	Anti-sense	5'aat cac tct cag cag cag cag g 3′	
Primer set II			
Ad-36 OF1	Outer Sense	5'gtc tgg aaa act gag tgt gga ta 3′	
Ad-36 OR1	Outer Anti-sense	5′ atc caa aat caa atg taa tag agt 3′	
Ad-36 IF2	Inner Sense	5′ tta act gga aaa gga ata ggt a 3′	~650 bp
Ad-36 IR2	Inner Anti-sense	5′ ggt gtt gtt ggt tgg ctt agg ata 3′	
Primer set III			
Beta-globin	Sense	5′ aca caa ctg tgt tca cta gc 3′	251 bp
	Anti-sense	5′ gga aaa tag acc aat agg ctg 3′	

2.5. Statistical analysis

Bio-statistical evaluation of the study results was conducted. Fischer's exact test was used to compare frequency and percentages for the patient and control group, and the Mann–Whitney U test was used for two group comparisons of continuous data and appropriate criteria of normal distribution. Logistic regression tests were performed according to the model of conditional forwarder for multivariate analysis. All analyses were performed using SPSS 21.0 package program (Licensed to Istanbul University). The significance value was considered as $p < 0.05$.

3. Results

Significant differences were detected between the patient and control group for serum cholesterol and triglyceride levels, but there was no significant differences for leptin and adiponectin levels. Ad-36 antibody positivity was detected with SNA in 6 of 49 (12.2%) obese adult patients, but no Ad-36 positivity was detected in the control group. A highly significant difference between the patient and control group for Ad-36 antibody positivity was found ($p < 0.05$) (Table 3).

In a comparison of cases with Ad-36 antibody and cases without Ad-36 antibody (Table 4), statistically significant differences in BMI ($p < 0.001$) (in two cases, BMI ≥ 40 kg/m^2), leptin ($p < 0.0001$), and adiponectin ($p < 0.0001$) levels were found. Mean BMI and leptin levels were found to be higher in the Ad-36 antibody positive group, while adiponectin levels were found to be lower in the Ad-36 antibody positive group. Although no statistically significant differences was found in serum cholesterol and triglyceride levels between the two groups ($p > 0.05$) (Table 4), lower levels of mean serum cholesterol and triglyceride levels were found in the Ad-36 antibody-positive patient group. Thus, similar results were detected in the high BMI group, as for the Ad-36 positive group.

In our study, the presence of the Ad-36 antibody and serum cholesterol and triglyceride levels were determined as a risk factor in multivariate analysis by logistic regression test of the parameters, including, for example, demographic and biochemical data, and the presence of Ad-36 ($p < 0.011$, $p < 0.002$, and $p < 0.0001$ respectively) (Table 5).

Using single-step in-house and nested-PCR with ATCC-VR-1610 as a positive control, Ad-36 DNA was not detected in any of the adipose tissue samples obtained from 49 obese adults (Fig. 2A and B). None of the adipose tissue samples from adults contained PCR inhibitors.

4. Discussion

Obesity, which is correlated with insulin resistance, diabetes, cardiovascular diseases (e.g., atherosclerosis and hypertension), and some types of cancers, is a world-wide known cause of serious morbidity and mortality, and has been purposed as a state of low-

Table 3
Test results of patient group and control group.

Characteristic/Variable	Patient group	Control group
Serum cholesterol (mmol/L)	166.4 ± 24.84	144.1 ± 42.23*
Serum triglyceride (mmol/L)	145.02 ± 34.23	94.53 ± 35.48*
Leptin (ng/ml)	15.71 ± 23.95	10.25 ± 8.021**
Adiponectin (μg/ml)	17.79 ± 9.906	22.42 ± 14.21**
Ad-36 Ab (positive/negative)	6/49	0/49*

Abbreviations: Ad-36 Ab: Adenovirus-36 Antibody; Mean ± sd: Mean ± standard deviation.
*$p < 0.05$; **$p > 0.05$.

Table 4
Comparison of BMI, leptin, adiponectin, cholesterol and triglyceride levels according to the presence of Ad-36 antibodies in the patient group.

	The presence of Ad-36 Ab		Statistical value
	Ad-36 Ab (+) (n = 6)	Ad-36 Ab (−) (n = 43)	
BMI (n:49)	38.67 ± 4.08	30.84 ± 1.21	$p < 0.001$
Leptin (n:49)	70.67 ± 33.1	8.042 ± 5.7	$p < 0.0001$
Adiponectin (n:49)	5.9 ± 0.63	19.45 ± 9.43	$p < 0.0001$
Serum cholesterol (n:49)	154.8 ± 22.8	168.0 ± 24.9	$p > 0.05$
Serum triglyceride (n:49)	137.7 ± 37.04	146.2 ± 34.12	$p > 0.05$

Table 5
Logistic regressions of variables.

Variables in the equation						
	B	S.E.	Wald	d.f.	Sig.	Exp (B)
Ad-36	−26.078	12862.396	0.000	1	0.011	0.000
Gender	−0.602	0.571	1.113	1	1.000	0.547
Age	0.005	0.024	0.042	1	0.182	1.005
Triglyceride	0.048	0.011	18.133	1	0.000	1.049
Cholesterol	−0.010	0.010	0.896	1	0.002	0.990
Adiponectin	−0.028	0.025	1.274	1	0.069	0.973
Leptin	−0.055	0.042	1.720	1	0.096	0.946
Constant	22.891	12862.396	0.000	1	0.992	8740502178

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.

grade chronic inflammation [24,25]. During the development and progression of obesity, inflammation and an altered immune response occur in adipose tissues, suggesting a close relationship between adipose tissue and immune system. It is known that several factors act together in the adipocyte–macrophage relationship, where molecules that interact synergistically and with a positive feed-back mechanism (cross-talk) play a role [26]. However, to enlighten the exact mechanism that initiates local adipose tissue inflammation leading to metabolic changes on the adipocytes is a crucial issue that has been severely under-researched. Recently, suggests that this mechanism may be related to infections, have led to the coining of a new term, “infectobesity”, as well as extensive sero-epidemiological studies, animal testing, and cellular experiments related to the role of Ad-36 in the pathological mechanism of obesity.

Preliminary evidence suggesting an Ad-36 and adipocyte differentiation relationship in the progression of obesity is based on animal studies by Dhurandhar et al. [12,13]. It has also been suggested that Ad-36 can infect primary human adipocytes derived from stem/stromal cells, as well as mouse, chicken, and monkey preadipocytes and adipocytes in these studies [12,13,20]. It is suggested that induction mechanism of adipocyte differentiation is initiated by the E4 (ORF1) viral gene which infects the host cell nucleus and that adipogenesis is consequently accelerated by progression of adipocyte proliferation and differentiation, and ultimately, cellular signaling pathways are affected [27–29]. Although the triggering role of Ad-36 in the Ad-36-obesity relationship is explained by these mechanisms, considering the replication potential and persistence of the virus in the host cell, other mechanisms may also impact the development and progression of obesity. Chronic inflammation process has been proposed to such a contributing mechanism [24,30].

In the study of Pasarica et al. [31], serum leptin levels of male Wistar rats infected with Ad-36, were found higher and corticosterone levels were sharply decreased in the infected rats. On the other hand, viral infections are generally known to be related with increases in inflammatory cytokines as interleukin-1 and they are also believed to stimulate the hypothalamus pituitary-adrenal axis to cause an increase in serum corticosterone levels [32]. Hovewer, Ad-36-induced supression corticosterone secretion may have several reasons. One of the plausible explanations about the effects of Ad-36 is a reduced inflammatory response of the Ad-36 or its effect on norepinephrine from the paraventricular nucleus. Consequently, Ad-36 contributes fat deposition, because of corticosterone prevents fat deposition, likely by decreasing glucose transport into adipocytes [33]. Although, our clinical study as a human study differs from Pasarica et al.'s study, our higher leptin results in group infected with Ad-36 against the control group were in concordance with the results of Pasarica et al.[31]. Na et al. [34] recently demonstrated in their mice model that cytokines were elevated in adipose tissue of Ad-36 infected mice within 3 days (MCP-1 and TNF-α), but that the reproductive capability of fat pads in adipocytes was increased after the 90th day. They suggested that Ad-36 infection stimulated an inflammatory state by increasing the level of MCP-1 through the nuclear factor κB, which in turn induced the infiltration of macrophages into adipocytes. This induced inflammation resulted in viral obesity, which caused chronic inflammation and affected lipid metabolism. In contrast to wild-type mice, MCP-1 knockout mice were protected from Ad-36

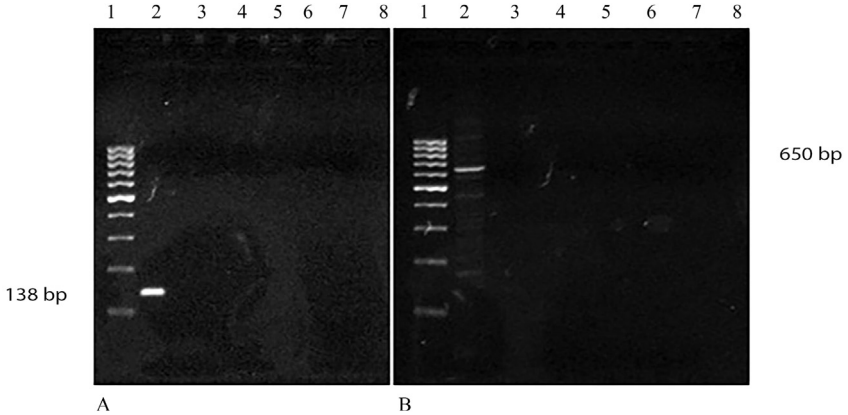

Fig. 2. A& B. Results of single-step in-house PCR and nested-PCR. A Lane 1: DNA ladder, 100 bp; lane 2: Single-step in-house PCR positive control, ~138 bp; lane 3–8: Some of the patient samples. B Lane 1: DNA ladder, 100 bp; lane 2: Nested-PCR positive control, ~650 bp; lane 3–8: Some of the patient samples.

induced inflammation and obesity. These results also suggest that MCP-1 plays a critical role in Ad-36-induced obesity. It is well determined that chronic inflammation play an important role in the progression of obesity. Studies indicate that adipocytes preserve their numbers and sizes under optimal conditions and as obesity progressing proinflammatory cytokines and chemokines were secreted by augmented adipocytes. Immune cells and macrophages infiltrate the adipocytes and remodeling of adipose tissue develops because of angiogenesis. The infiltrated macrophages secrete IL-6, IL-8, TNF-α and MCP-1 and adipocytes lose homeostasis of the free acid pathway during the inflammatory state [30].

According to the studies examining this relationship, Atkinson et al. [16] suggested a strong link between Ad-36 and obesity by detecting Ad-36 antibodies with SNA in 30% and 11% of obese and non-obese adults, respectively but they also reported that serum cholesterol and triglyceride levels were paradoxically decreased in obese patients. The relationship between Ad-36 and obesity was also reported in similar studies [17,19,35]. Despite these studies, there are also contradictory studies. Na et al. [36] couldn't find a significant difference between obese, overweight, and normal individuals for Ad-36 antibodies with SNA. They also detected lower triglyceride levels in the three groups and higher total cholesterol levels only in the obese group. In another study conducted in a military unit of the USA, Ad-36 antibodies were detected in 34% of obese and 39% of non-obese individuals, and cholesterol and triglyceride levels were detected at higher levels in obese individuals compared to the control group [37]. According to a cross-sectional based study of 509 individuals performed in Europe by Goossens et al. [38] using SNA, Ad-36 antibodies were found in only 5.7% of obese and 3.9% of non-obese individuals, suggesting that there is no relationship between Ad-36 and obesity.

Ad-36 antibody titers were detected at significantly higher levels in obese children than non-obese children for the first time in Turkey by Cakmakliogullari et al. [11]. Although, their study was the first study to be conducted in a pediatric group, our study differs from their study in various aspects (e.g., investigation of Ad-36 antibody levels by SNA in obese adults and Ad-36 DNA detection in adipose tissue of obese adults).

We detected a highly significant difference between obese and non-obese groups for the presence of the Ad-36 antibody by univariant analysis ($p < 0.001$). We also detected, the presence of Ad-36 antibody, cholesterol, and triglyceride levels as independent risk factors according to the multivariate analysis ($p < 0.011$, $p < 0.002$, $p < 0.0001$, respectively). In the adipose tissue samples obtained, no Ad-36 DNA was detected. While our serological results show similarities with the serologic-based studies performed in the USA and Europe [8,16,17,35], our result of no detectable Ad-36 DNA in human adipose tissue samples differed from the results of Pasarica et al. [20] and Salehian et al. [21]. However, our results showed similarity with the study of Goossens et al. [38].

In our study, the obese cases with positive Ad-36 antibodies (i.e., obese cases with high BMI) had significantly higher mean levels of leptin compared to the obese cases with negative Ad-36 antibodies ($p < 0.001$ and $p < 0.0001$ respectively, according to the univariate analysis). This result was in concordance with the high leptin result of pediatric obese group in the study of Cakmakliogullari et al. [11]. Mean levels of adiponectin were found to be significantly lower ($p < 0.001$) in the obese cases with positive Ad-36 antibodies than in the obese cases with negative Ad-36 antibodies. Our result was in concordance with the study results of Jiao et al. [10]. Atkinson et al. [16] reported that serum cholesterol and triglyceride levels were paradoxically decreased in obese patients, although not statistically significant, lower mean levels of serum cholesterol and triglycerides were detected in the obese cases with positive Ad-36 antibodies according to the univariate analysis in our study ($p > 0.05$).

The detection of significantly higher BMI and leptin, lower adinopectin, and paradoxically insignificantly lower levels of serum cholesterol and triglyceride levels in the obese group with positive Ad-36 antibodies, as compared to the obese group with negative Ad-36, showed similarity with the animal model studies of Dhurandhar et al. [12,13,15] and with the human studies of Atkinson et al. [16] and Jiao et al. [10].

However, in line with the relationship between morbid obesity and Ad-36 level described by Almgren et al. [6], even less number, we detected BMI $\geq$ 40 kg/m^2 in two Ad-36 antibody positive cases of our study.

Even though, both Almgren et al. [6] and Aldhoon-Hainerova et al. [7] found a strong correlation in women for Ad-36-obesity relationship, we couldn't find a significant difference between women and men.

In elucidating the similarity between our data and the aforementioned studies, as well as the pathophysiological processes at work, it has been suggested that a decrease in peripheral serum cholesterol and triglyceride levels is observed due to an increase in lipid storage, with the activation of inflammatory processes coupled with alterations in lipid metabolism (e.g., hyperplasia and hypertrophy of adipose tissues) seen in obese individuals infected with Ad-36 [16,20,36,39,40]. In this first reported clinical study from Turkey focusing on the Ad-36-obesity relationship in obese adults, however, we could not detect Ad-36 DNA in adipose tissue cells.

In conclusion, our results are mostly in line with those of other international studies on the Ad-36-obesity relationship. Our results, similar to other studies, demonstrate detection of significantly higher Ad-36 antibodies in obese patients when compared to non-obese patients in both univariant and multivariant analysis. Other data generated on this study (especially on leptin, adiponectin, serum cholesterol and triglyceride levels) also show similarities to data obtained in these international studies.

Here, based on our results, we suggest that Ad-36 may have a role in obesity. Ultimately, we need new and extended serial studies, particularly cohort and human based experimental studies, in order to gain a better understanding of the Ad-36-obesity relationship.

Conflict of interest

The authors have declared that no competing interests exist.

Acknowledgments

This study was supported by the Research fund of the Istanbul University, project number: 23874. We are grateful to the Dr. Adam Lipson (MD and PHD), Philadelphia and Dr. Sami Sarıbaş (PHD) Temple Medical School, Philadelphia and American Manuscript Editors, an affiliate of Grammar Labs, LLC, Seattle, Washington, USA to revise English language of the manuscript. We are also thank Professor Richard L. Atkinson for advices about the methods of the study, Virginia Commonwealth University; Director, Obetech Obesity Research Center 800 East Leigh St., Suite 50 Richmond, USA.

References

[1] N.F. Butte, E. Christiansen, T.I. Sorensen, Energy imbalance underlying the development of childhood obesity, Obesity 15 (2007) 3056–3066.
[2] World Health Organization. Obesity and Overweight Fact Sheet No: 311, Geneva, WHO, http//www.who.int/mediacentre/factsheets/fs311/en/.
[3] N.V. Dhurandhar, Infectobesity: obesity of infectious origin, J. Nutr. 131 (2001) 2794–2797.
[4] N.V. Dhurandhar, P. Kulkarni, S.M. Ajinkya, A. Sherikar, Avian adenovirus

leading to pathognomic obesity in chicken, J. Bombay Vet. Coll. 2 (1990) 131–132.
[5] N.V. Dhurandhar, P. Kulkarni, S.M. Ajinkya, A. Sherikar, R.L. Atkinson, Association of adenovirus infection with human obesity, Obes. Res. 5 (1997) 464–469.
[6] M. Almgren, R. Atkinson, J. He, A. Hilding, A. Hagman, A. Wolk, et al., Adenovirus-36 is associated with obesity in children and adults in Sweden as determined by rapid ELISA, PLoS One 7 (7) (2012) e41652.
[7] I. Aldhoon-Hainerova, H. Zamrazilova, R.L. Atkinson, L. Dusatkova, B. Sedlackova, P. Hlavaty, Clinical and laboratory characteristics of 1179 Czech adolescents evaluated for antibodies to human adenovirus 36, Int. J. Obes. (Lond.) 38 (2) (2014) 285–291.
[8] I. Parra- Rojas, O. Del Moral-Hernández, A.B. Salgado-Bernabé, I.P. Guzmán-Guzmán, L. Salgado-Goytia, J.F. Muñoz-Valle, Adenovirus-36 seropositivity and its relation with obesity and metabolic profile in children, Int. J. Endocrinol. 2013 (2013) 463194.
[9] I. Bil-Lula, S. Stapor, M. Sochocka, M. Wolyniec, K. Zatońska, R. Ilow, et al., Infectobesity in the Polish population-evaluation of an association between adenoviruses type 5, 31, 36 and human obesity, Int. J. Virol. Mol. Biol. 3 (1) (2014) 1–8.
[10] Y. Jiao, X. Mao, X. Chang, K. Abudureyimu, C. Zhang, J. Lu, et al., Adenovirus36 infection expresses cellular APMI and visfatin genes in overweight Uygur individuals, Diagn. Pathol. 9 (2014) 83.
[11] E.K. Cakmakliogullari, T. Sanlidag, B. Ersoy, S. Akcali, A. Var, C. Cicek, Are human adenovirus-5 and 36 associated with obesity in children? J. Investig. Med. 62 (5) (2014) 821–824.
[12] N.V. Dhurandhar, B.A. Israel, J.M. Kolesar, G. Mayhew, M.E. Cook, R.L. Atkinson, Increased adiposity in animals due to a human virus, Int. J. Obes. Relat. Metab. Disord. 24 (2000) 989–996.
[13] N.V. Dhurandhar, L.D. Whigham, D.H. Abbott, N.J. Schultz-Darken, B.A. Israel, S.M. Bradley, et al., Human adenovirus Ad-36 promotes weight gain in male rhesus and marmoset monkeys, J. Nutr. 132 (10) (2002) 3155–3160.
[14] M. Pasarica, S. Loiler, N.V. Dhurandhar, Acute effect of infection by adipogenic human adenovirus Ad36, Arch. Virol. 153 (2008) 2097–2102.
[15] N.V. Dhurandhar, B.A. Israel, J.M. Kolesar, G. Mayhew, M.E. Cook, R.L. Atkinson, Transmissibility of adenovirus-induced adiposity in a chicken model, Int. J. Obes. Relat. Metab. Disord. 25 (7) (2001) 990–996.
[16] R.L. Atkinson, N.V. Dhurandhar, D.H. Allison, R.L. Bowen, B.A. Israel, J.R. Albu, A.S. Augustus, Human adenovirus-36 is associated with increased body weight and paradoxical reduction of serum lipids, Int. J. Obes. (Lond.) 29 (2005) 281–286.
[17] R.L. Atkinson, I. Lee, H.J. Shin, J. He, Human adenovirus-36 antibody status is associated with obesity in children, Int. J. Pediatr. Obes. 5 (2) (2010) 157–160.
[18] H.N. Na, Y.M. Hong, J. Kim, H.K. Kim, I. Jo, J.H. Nam, Association between human adenovirus-36 and lipid disorders in Korean schoolchildren, Int. J. Obes. (Lond.) 34 (1) (2010) 89–93.
[19] C. Gabbert, M. Donohue, J. Arnold, J.B. Schwimmer, Adenovirus-36 and obesity in children and adolescents, Pediatrics 126 (4) (2010) 721–726.
[20] M. Pasarica, N. Mashtalir, E.J. Mc Allister, G.E. Kilroy, J. Koska, P. Permana, et al., Adipogenic human adenovirus Ad-36 induces commitment differentiation and lipid accumulation in human adipose derived stem cells, Stem Cells 26 (2008) 969–978.
[21] B. Salehian, S.J. Forman, F.R. Kandeel, D.E. Bruner, J. He, R.L. Atkinson, Adenovirus 36 DNA in adipose tissue of patient with unusual visceral obesity, Emerg. Infect. Dis. 16 (5) (2010) 850–852.
[22] A.K. Tosh, A. Broy-Aschenbrenner, J. El Khatib, B. Ge, Adenovirus-36 antibody status & BMI comparison among obese Missouri adolescents, Mo Med. 109 (5) (2012) 402–403.
[23] L.J. Reed, H. Muench, Simple method for estimating 50% end points, Am. J. Hyg. 27 (1938) 493–497.
[24] G.S. Hotamisligil, Inflammation and metabolic disorders, Nature 444 (2006) 860–867.
[25] U.J. Jung, M.S. Choi, Obesity and its metabolic complications: the role of adipokines and the relationship between obesity, inflammation, insulin resistance, dyslipidemia and nonalcoholic fatty liver disease, Int. J. Mol. Sci. 15 (2014) 6184–6223.
[26] F.P. Heredia, S.G. Martinez, A. Marcos, Chronic and degenerative diseases Obesity, inflammation and the immune system, Proc. Nutr. Soc. 71 (2012) 332–338.
[27] J.J. Arnold, M. Janoska, A.E. Kajon, D. Metzgar, N.R. Hudson, S. Torres, et al., Genomic characterization of human adenovirus 36, a putative obesity agent, Virus Res. 149 (2) (2010) 152–161.
[28] S.D. Vangiparum, J. Sheele, R.L. Atkinson, T.C. Holland, N.V. Dhurandhar, A human adenovirus enhances preadipocyte differentiation, Obes. Res. 12 (5) (2004) 770–777.
[29] P.M. Rogers, K.A. Fusinski, M.A. Rathod, S.A. Loiler, M. Pasarica, M.K. Shaw, et al., Human adenovirus Ad-36 induces adipogenesis via its E4 orf-1 gene, Int. J. Obes. (Lond.) 32 (3) (2008) 397–406.
[30] H.N. Na, J.H. Nam, Infectobesity: a new area for microbiological and virological research, J. Bacteriol. Virol. 41 (2) (2011) 65–76.
[31] M. Pasarica, A.C. Shin, M. Yu, H.M.U. Yang, M. Rathod, C. Jen, et al., Human adenovirus 36 induces adiposity increases insulin sensitivity, and alters hypothalamic monoamines in rats, Obesity 14 (11) (2006) 1905–1913.
[32] C.A. Dinarello, The pathophysiology of the pro-inflammatory cytokines, Biotherapy 2 (1990) 189–191.
[33] J. Griffin, S. Ojeda, in: J. Griffin, S. Ojeda (Eds.), Textbook of Endocrine Physiology, Oxford University Press, New York, 1988.
[34] H.N. Na, J.H. Nam, Adenovirus 36 as an obesity agents maintains the obesity state by increasing MCP-1 and inducing inflammation, J. Infect. Dis. 205 (2012) 914–922.
[35] G.M. Trovato, A. Castro, A. Tanzuso, A. Garozzo, G.F. Martines, C. Pirri, et al., Human obesity relationship with Ad36 adenovirus and insulin resistance, Int. J. Obes. (Lond.) 33 (2009) 1402–1409.
[36] H.N. Na, J. Kim, H.S. Lee, K.W. Shim, H. Kim, S.H. Jee, et al., Association of human adenovirus-36 in overweight Korean adults, Int. J. Obes. (Lond.) 36 (2) (2012) 281–285.
[37] M.P. Broderick, C.J. Hansen, M. Irvine, D. Metzgar, K. Campbell, C. Baker, et al., Adenovirus 36 seropositivity is strongly associated with race and gender, but not obesity, among US military personnel, Int. J. Obes. (Lond.) 34 (2) (2010) 302–308.
[38] V.J. Goossens, S.A. De Jager, G.E. Grauls, M. Gielen, R.F. Vlietinck, C.A. Derom, et al., Lack of evidence for the role of human adenovirus-36 in obesity in a European cohort, Obesity 19 (1) (2011) 220–221.
[39] D.B. Hausman, M. DiGirolamo, T.J. Bartness, G.J. Hausman, R.J. Martin, The biology of white adipocyte proliferation, Obes. Rev. 2 (2001) 239–254.
[40] M.S. Desruisseaux, Nagajyothi, M.E. Trujillo, H.B. Tanowitz, P.E. Scherer, Adipocyte, adipose tissue and infectious disease, Infect. Immun. 75 (3) (2007) 1066–1078.