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Original Article

Adenovirus-36 DNA in Adipose Tissue of Iranian Patients with Severe Obesity and A Paradoxical Alteration of Serum Lipids Due to the Virus

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Abstract

Background and Aims: Obesity is one of the most important health problems today that causes other diseases such as diabetes and heart diseases. Obesity is caused by a variety of factors, and viruses, especially adenoviruses type 36. This study was performed to detect the adenovirus 36 genome in the adipose tissue of patients who underwent surgery due to severe obesity. Also, the prevalence of antibodies against adenovirus 36 in the control and patient groups was compared and their biochemical parameters were compared and analyzed.

Materials and Methods: Adenovirus genome was detected in patients with abdominal adipose tissue by PCR technique. Positive samples were sequenced and a phylogenetic tree was drawn. Prevalence of specific antibodies against adenovirus 36 in case and control groups was determined by ELISA technique.

Results: The adenovirus genome was detected in the adipose tissue in 4 of the 60 samples in the case group. 43 obese individuals with a body mass index of less than 35 were also selected as the control group. The prevalence of antibodies against this virus in 103 psrsons was 49%. The prevalence of antibodies was 33% in the patient group and 15% in the control group. In people with antibodies to adenovirus 36, the serum lipid profile changed significantly. These people had lower serum triglycerides and higher low density lipoprotein .Finally, the sequencing analysis showed the adenoviruses found in the specimens were adenovirus-36 and are belonged to subgroup D.

Conclusion: Adenovirus 36 is detected in the adipose tissue of severely obese individuals and the presence of its antibody in the serum affects the lipid profile.

Keywords: Adenovirus type 36; obesity; adenovirus; adipogenic viruses

Introduction

Adenoviruses are members of the *Adenoviridae* family which are non-enveloped, icosahedral, and doubled stranded DNA viruses. Adenoviruses are associated with respiratory, gastrointestinal, and conjunctival infections (1). Obesity is one of the major problems in industrialized and advanced societies today (2). The successful manage-ment of this important medical condition requires a good knowledge of the factors contributing to its development (2). The etiology of obesity is multifactorial (3). Previous studies have shown that obesity can be associated with adenoviral infection. Dhuranhar et al, first reported the association between adenovirus 36 (Ad-36) and obesity (4). The two human adenoviruse, Ad-37, and Ad-5 cause obesity in animals (5,6). These three adenoviruses affect adipocytes directly. These viruses stimulate enzymes and transcription factors which causes accumu-lation of triglycerides and differentiation of preadipocytes into mature adipocytes (7). Also, the obesogenic avian adenovirus, SMAM-1 is the

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only animal virus which causes obesity in human (8).

Ad-36 was first isolated from the faeces of a girl with enteritis in 1978. The virus belongs to subgroup D (9). Dhurandar et al. revealed that mice and chicken infected with Ad-36 showed increase in body weight due to fat accumulatio (4). These obese animals had low serum levels of total cholesterol and triglycerides (4). In the other study, Pasarica et al, found the rats which infected with Ad-36, showed increase in body weight, in insulin sensitivity and glucose uptake (10). Also, the results of a study of hamsters revealed that Ad-36 is associated with decreased triglycerides level, but increases the low-density lipoprotein cholesterol which may increase the risk of cardiovascular disorders (11). On the other hand, Dhurandhar et al, showed that AD-36 infection could be transmitted from one infected chicken to another chicken in the same cage and inoculation of blood from AD-36 infected animals to uninfected animals causes the recipients became obese (12). Another studies were carried out to evaluate the presence or/and level of anti-AD-36 antibodies in the blood of obese and normal persons, and correlate them with body mass index(BMI), Triglyceride (TG) and cholesterol (Chol) levels. Some studies have indicated a strict correlation between seroconversion against AD-36 and increased BMI and altered lipid metabolism, but others have different conclusions. One study in the USA revealed a significant association between obesity and positive AD-36 antibody status. In this research, the presence of AD-36 antibodies in the obese persons was 30%, whereas 11% of the non-obese persons were seropositive (13). Similar results have been reported in Italy by Trovato et al. They found that seroconversion against AD-36 in obese and normal persons was 64% and 32%, repectively. Also, BMI, blood pressure, and TG levels were significantly higher in the seropositive group (14). On the other hand, one study in Korea showed the always≥30% prevalence of AD-36 antibody positively in normal or obese adults (15). Raben et al. reported a very low prevalence of serocoversion against AD-36 in both obese and normal persons in Denmark (5.7% vs. 10.0%) (16). Guoossens et al. found a similarly very low prevalence of seropositivity for AD-36 antibodies in the obese and normal persons(5.7% vs. 3,9%) (17).

They also found no adenoviral DNA in the visceral adipose tissue of 31 severely obese patients. In comparison with those of the adult studies, the results relating to children are more similar to the results of animal studies.18,19 In these studies, the obese children which were seropositive, had higher TG and Chol levels (18,19).

This study was designed to evaluate the prevalence of antibodies against AD-36 in the serum of obese patients referring to Rasul-e Akram Hospital, Tehren compared to control group. Then the presence of viral DNA in abdominal adipose tissue of obese patients who had undergone surgery.

This project was approved by the Code of Ethics of Iran University of Medical Sciences.

Methods

Sampling: 60 adult obese individuals with a BMI greater than 35 who were candidates for surgery were selected as the case group.

Abdominal visceral fat was sampled during surgery. Forty-three adults with a BMI of less than 35 were selected as the control group.

Serum samples were taken from both groups. Fasting blood sugar, triglyceride and total blood cholesterol, HDL, LDL and glycosylated hemoglobin(HbA1C) were tested for both groups.

ELISA for Antibodies Against AD-36: This evaluation was carried out using commercially available Enzyme linked immunosorbent assay (ELISA) kits (human adenovirus-36 Ab IgG kit, MyBiosource, USA), according to manufacturer's recommendations.

The concentrations of antibodies in each specimen were determined by measuring the absorbance at 450 nm using a microplate reader (Hiperion MPR 4+, Germany). The positive serum control must have an optical density (OD450) reading greater than 1.00.

Table 1. Comparison of biochemical parameters in the two groups of AD-36 seropositive and seronegative participants

Parameter	Seronegative	Seropositive	p-value
Age	41 (13%)	40 (8%)	0.5
Sex	Female, 56 (74%) Male, 20 (26%)	Female, 18 (67%) Male, 9 (33%)	0.6
FBS	96	108	0.12
HbA1C	5.40	5.90	0.05
TG	87	115	≤0.001
Chol	164	178	0.13
HDL	54	41	≤0.001
LDL	111	120	0.3

The Cut off value was equal to average negative control value + 0.15. Therefore, an OD450 equal or greater than this value was scored as positive.

DNA Extraction: DNA extraction was performed by Viral Nucleic Acid Extraction Kit (Yekta Tajhiz Azma, Iran).

The concentration of the extracted DNA was determined using NanoDrop-1000 (Thermo Scientific, USA).

Human adenovirus detection by PCR: PCR was used to amplify a 243 bp region of the hexon gene. The forward and reverse primers were 5'-GCTTCGGAGTACCTGAGYCC-3' and 5'-GGCCATRTCCAGCACTCKGT-3' , repectively. PCR was performed in a thermal cycler machine. The total volume of the reaction mix was 20 µL, and it contained the following components: 10µL of master mix (Yekta Tajhiz Azma, Iran), 0.5 µL of each primer, 3µL of template, and 6 µL of double-distilled water (ddH2O). Thermal PCR cycles were as follows: The initial denaturation step was carried out at 95°C for 5 minutes, followed by 35 cycles of 95°C (30 seconds), 60°C (30 seconds), and 72°C (30 seconds), and an extension of 72°C for 5 minutes. PCR products were separated on a 1.5% agarose gel stained with safe stain and visualized under UV light.

Sequencing and phylogenetic tree: The PCR products were sequenced directly by Sanger sequencing. The obtained sequences were aligned with the HAdV reference sequence by the CLC Main Workbench 5.5 software (CLC bio, Boston, MA, USA). The phylogenetic analysis was performed using MEGA version 10.

The phylogenetic tree was constructed using the Tamura three-parameter model with a bootstrap test of 100 replicates .

Statistical Analysis: Statistical analysis was performed using SPSS version 22 software (SPSS Inc., Chicago, IL). The Chi-squared test was used to assess the statistical differences between groups. A P-value of less than 0.05 was considered statistically significant.

Results

Demographic characteristics: Out of 103 participants included in the study, 74 (72%) were females and 29 (28%) were males with mean age of 41 years . The mean age in case and control groups was 36 and 48, respectively, and the results of the independent sample t-test showed that this difference was statistically significant (p-value< 0.001).

According to Chi-square test results, these two groups were not statistically significant different in terms of sex distribution (p-value = 0.5).

Analysis of IgG against adenovirus-36: Among 103 participants, 27 (49%) were positive for anti-AD-36 IgG. Positivity percentages of IgG detection in case and control groups were 33% and 15%, respec-tively (p-value = 0.069). The results showed the AD-36 antibody-positive partici-pants had significantly higher serum TG levels.

Also, they had a significant shift from high-density lipoprotein to low- density lipoprotein choles-terol. Table 1 shows a comparison of bioche-mical parameters in the two groups of seropositive and seronegative participants.

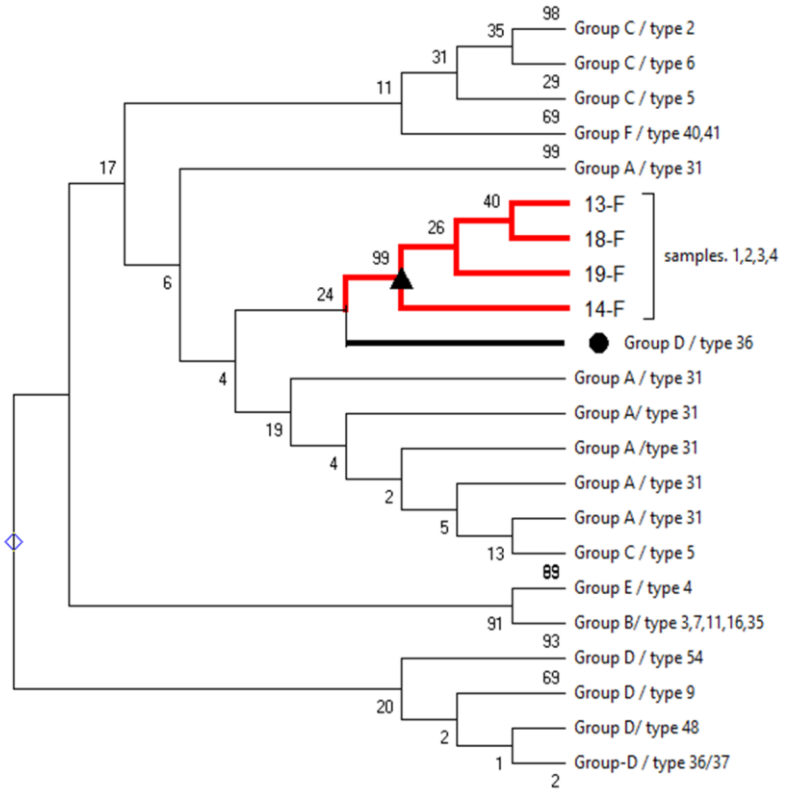

Fig. 1. The phylogenetic tree of 4 isolated adenoviruses. This analysis that the adenoviruses found in the specimens were adenovirus-36 and are belonged to subgroup D.

Adenovirus DNA detection in adipose tissue specimens: For the detection of adenovirus in adipose tissue specimens, PCR assay was used. The results showed that adipose tissue in 4 of the 60 samples in the case group contained the adenovirus genome.

Analysis of the sequence alignment: Positive samples obtained from PCR test were examined to determine the type of adenovirus. After sequencing, the samples were analyzed using CLC work bench software and sequence trimming. The type of each positive case was determined using BLAST tool in NCBI database and alignment with different types of adenovirus. The results of this study showed that the adenoviruses found in the specimens were adenovirus-36. Sequences obtained using MEGA software version 10 along with sequences related to some isolates of other studies that represented different types of virus were aligned with the CLUSTAL W method. Items aligned using the Neighbor joining method with bootstrap 1000 were used to design the phylogenetic tree. The phylogenetic tree is shown in the Fig.1.

Discussion

Obesity is an important medical problem and one of the underlying factors of other diseases (20). Obesity is caused by various factors and viruses are one of the various factors that are involved in the mechanism of obesity (21). Various viruses in humans and animals are associated with obesity. For example, Canine distemper virus, Rous-associated virus and Borna diseae virus cause obesity in animals, but the most important obesity-related viruses are from the adenovirus family. Human adenoviruses type 5 and type 37 are associated with obesity in animals. A type of avian adenovirus called SMAM-1 is associated with obesity in humans, and most importantly, human adenovirus type 36, which is the only

human virus associated with obesity in humans (22-24).

Various mechanisms have been identified for viruses to cause obesity. In Canine distemper virus, for example, damage to the hypothalamus causes obesity and increases serum triglycerides and cholesterol (25). In Rous-associated virus type 7, chickens also become obese due to damage to the central nervous system (26).

Adenovirus 36 increases lipogenic enzymes in adipose tissue. This mechanism is influenced by the E4ORF1 gene of the virus. Thus, triglyceride storage and differentiation of progenitor cells into new fat cells increases (27,28). Adenoviruses 36 and 37 inhibit leptin expression in infected cells, causing fat to accumulate in adipocytes. Adenovirus 36 also increases appetite by lowering epinephrine level (29). Adenovirus 36 also affects the endocrine system and causes obesity by affecting corticosterone secretion (30). The virus increases inflammation in adipose tissue by increasing MCP-1, IL-6, TNF-α and NFκB, causing permanent obesity in the body (31,32).

The prevalence of antibodies against this virus has been studied in different countries. Also, the relationship between the presence of antibodies and obesity has been investigated and changes in lipid profile in the serum of individuals have been studied. In many studies, the increased prevalence of virus antibodies is associated with the obesity in individuals, but its effect on serum lipids varies in different studies. In adults, the presence of antibodies is usually associated with an increase in body mass index, and serum triglycerides and cholesterol are reduced. Also, in some studies, the presence of antibodies is not related to obesity, but changes in serum lipids may occur. In children, the presence of antibodies is associated with an increase in body mass index, and serum triglycerides and cholesterol are increased (33).

The present study was performed on case and control groups. Both groups contain adult and obese subjects. In the case group of 60 people, the body mass index was higher than 35 and they underwent surgery due to severe obesity. Abdominal visceral fat was obtained from this group during surgery. Adenovirus genome was detected in 4 patients by PCR method. After sequencing and drawing of phylogenetic tree, it was determined that all 4 samples are adenoviruses of D group and possibly AD-36.

Few studies have been performed on adipose tissue in obese individuals.

In 2010, Salehian et al. examined the presence of AD-36 DNA in adipose tissue samples from obese individuals in the United States. Out of 12 samples in this study, 5 samples had a positive result by PCR (34).

In 2012, Atkinson Rl et al. Examined the prevalence of adenotype 36 in obese individuals in the Netherlands. In this study, out of 31 adipose tissue samples, 25 samples contained virus DNA (30).

In the present study, an ELISA test was performed to detect antibodies against adenovirus 36 in case (60) and control (43) groups. Among 103 participants, 27 (49%) were positive for anti-AD-36 IgG. Positivity percentages of IgG detection in case and control groups were 33% and 15%, respecti-vely.

The AD-36 antibody-positive participants had significantly higher serum TG levels. Also, they had a significant shift from high-density lipoprotein to low- density lipoprotein cholesterol.

Ponterio and colleagues examined the association of adenotype 36 with weight gain in Italy in 2015. In this study, the prevalence of antibodies in obese and non-obese individuals was determined in 502 samples using the ELISA technique. The results of this study showed that antibodies against type 36 were found in 30% of obese and 11% of non-obese people (8,30).

Shohreh Ehsandar and her colleagues studied the prevalence of human adenovirus type 36 in Tehran in 2014 and its relationship with obesity and lipid factors. Despite the high prevalence of adenovirus type 36, there was no significant relationship between presence of antibody and body mass index, but the presence of the antibody was associated with lipid disorders (35).

In 2016, Fatemeh Shirani et al. in Ahvaz studied the correlation between prevalence of adenovirus type 36 antibodies and obesity. The

study was performed on 70 samples. The antibody level of obese persons was higher than those of non obese persons (30% vs 11%) but serum total cholesterol and triglycerides levels are significantly lower (36).

Atkinson and colleagues in the United States in 2005 examined adenovirus 36 for its prevalence of antibodies in obese and non-obese individuals, of whom 348 were 294 over 18 years old and 54 were under 18 years old. In children and adolescents, the association between adeno 36 and obesity was significant. Serum levels of triglycerides and total cholesterol in obese individuals with type 36 antibodies were lower than those of negative antibodies (13).

In the present study, the prevalence of antibodies in the case group was higher than the control group, although the difference was not significant and could be due to the fact that the control group is also obese, but their body mass index is lower. On the other hand, the result of this study on lipid parameters is inconsistent with previous studies because in this case group, serum triglyceride decreases but LDL level increases, which is associated with a higher risk of heart diseases. In the case group of severely obese individuals, the effect of adenovirus on adipose tissue may be to produce new fat cells in the tissues, which in turn reduces serum triglycerides, but increased LDL level in these people increases heart diseases. Also, the differences observed in different studies can be due to geographical and genetic differences.

Studies have shown that adenoviruses 36 and 37 in animals increase cellular uptake of glucose in adipose and muscle tissue and reduce glucose accumulation in liver tissue and insulin resistance (36). Therefore, they could be a new target for the treatment of insulin-resistant diabetes, especially adenovirus 37, which decreases glucose in mice but does not contribute to obesity in mice.

These adenoviruses could be used to explain the pathophysiology of some obesity cases and be a new model for treating obesity-related diabetes.

Conclusion

Adenovirus 36 is detected in the adipose tissue of severely obese individuals and the presence of its antibody in the serum affects the lipid profile.

Acknowledgment

None.

Conflict of interest

No conflict of interest is declared.

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