

Determination of Adenovirus 5 and 37 seropositivity in obese patients

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Abstract

Obesity is seen as one of the most important health problems of our age due to its accompanying diseases such as diabetes, high blood pressure and cholesterol. It is an epidemic that started in the 1980s and continues worldwide, regardless of the level of development of the countries, and is associated with many factors. In the face of the rapidly increasing prevalence of obesity, it has been thought whether microorganisms can play a role in the formation of obesity. Based on this idea, the concept of 'infectobesity' was introduced and studies on this subject have shown that some microorganisms cause obesity in experimental animal models such as mice, chickens and non-human primates. One of the microorganisms thought to cause obesity is Adenoviruses. Especially Adenovirus-36 (Ad36), Adenovirus-5 (Ad5) and Adenovirus-37 (Ad37) serotypes are thought to have effects on fat regulation in the body. There are very few studies investigating the relationship between Ad5 and Ad37 serotypes and obesity in humans. The aim of our study is to increase the research on this subject, to develop a new perspective on the infectious etiology of obesity, and to contribute to the development of new treatment approaches for obesity and obesity-related diseases.

INTRODUCTION

Obesity is considered one of the most significant health problems of our time due to its accompanying diseases such as diabetes, hypertension, and high cholesterol (1). It started in the 1980s and has continued worldwide regardless of the countries' levels of development, being associated with various factors. Among these factors are environmental, genetic, endocrine factors, nutrition, and lifestyle (2). Despite the emphasis on nutrition, changes in nutrition and lifestyle have not been seen to completely solve the problem.

In the face of the rapidly increasing prevalence of obesity, the role of microorganisms in the formation of obesity has been considered (3). Based on this idea, the concept of "infectobesity" has been put forward, and studies on this subject have shown that some microorganisms can cause obesity in animal models such as mice, chickens, and non-human primates. Since 1982, viruses associated with obesity have been identified, including Canine distemper virus (CDV), Rous-associated virus-7 (RAV-7), Borna disease virus (BDV), Scrapie agent prion, and SMAM-1 from animal viruses; and from human viruses, only Adenovirus 36 (Ad-36), Adenovirus 37 (Ad-37), and Adenovirus 5 (Ad-5) from the Adenoviridae family (4).

Adenoviruses are non-enveloped, icosahedral symmetric, and medium-sized viruses. All adenoviruses that infect humans share the same common genome; they contain a single linear and double-stranded DNA molecule. There are more than 50 serotypes infecting humans (5).

Adenoviruses associated with obesity are believed to cause triglyceride accumulation in fat cells (adipocytes) and differentiation of immature adipocytes (pre-adipocytes) into mature adipocytes (3). It has been determined that Ad-5 and Ad-37 increase the formation of fat cells in animals and contribute to the development

of obesity. Ad-36 is the most studied human adenovirus to date, and its association with human obesity has been proven (6).

Leptin hormone is secreted by adipocytes of adipose tissue, reduces appetite, increases energy expenditure, stimulates the breakdown of fat cells, and inhibits lipid synthesis. It is known that insufficient synthesis of leptin leads to more fat storage. To investigate the mechanism of adipose tissue formation associated with adenovirus, the secretion of leptin in human pre-adipose cells infected with Adenoviruses was examined. It was found that in cells infected with Ad-37, leptin mRNA synthesis was suppressed, and lipid accumulation increased simultaneously. Additionally, changes in enzymes such as intracellular glycerol 3-phosphate dehydrogenase (GPDH) induced the transformation of pre-adipocytes into mature adipocytes (3).

The aim of our study is to investigate the seropositivity of Adenovirus 5 and 37 in blood samples taken from 48 obese patients and 42 normal weight individuals without underlying chronic diseases, not using any medication routinely, and without immune system problems, and whether there is a significant difference between obese and normal weight individuals. Increasing the number of studies on this subject and developing a new perspective on the infectious etiology of obesity will contribute to developing new treatment approaches for obesity and obesity-related diseases.

MATERIALS AND METHODS

This study was conducted with the approval of the Istanbul Aydin University Non-Interventional Clinical Research Ethics Committee with decision number 2023/16. Study groups were formed based on volunteerism, and individuals aged between 18-60 years without any chronic diseases and immune deficiencies were selected for obese and control groups. For obese patients, individuals with BMI ≥ 30 and for healthy groups, individuals with BMI greater than 18.5 and less than 25 were selected. 10 ml of fasting blood was taken from 48 patients and 42 healthy individuals who met these criteria. The collected blood samples were centrifuged to separate the sera and stored at -20°C until the study was conducted.

The seropositivity of Ad-37 and Ad-5 in the collected study samples was investigated using ELISA test kits, which can demonstrate antigen-antibody reactions through enzymes. The serum samples of obese and healthy individuals were tested according to the instructions of the test kits. Optical density measurements at a wavelength of 450 nm were made using the Biotek-Epoch brand ELISA microplate reader.

The obtained results were interpreted as positive or negative by taking the average of the standard samples included in the ELISA kits and accepting it as the cutoff value. Normality analyzes were examined to analyze the data, and it was observed that the data were not normally distributed. Independent two-group Mann-Whitney U test, a non-parametric analysis, was used to determine the statistical significance of the results and the relationship between them, and a significance level of $p < 0.05$ was accepted.

RESULTS

The average age of the patient group from which the samples were collected was found to be 39.69 ± 12.17 , and the average age of the control group was 24.71 ± 7.79 . In the patient group, there were 20 females with an average age of 42.35 ± 10.47 and 28 males with an average age of 37.65 ± 13.16 . In the control group, there were 26 females with an average age of 24.84 ± 6.19 and 16 males with an average age of 24.5 ± 10.10 . The physical characteristics of the patient and control groups are shown in Table I.

Table I. Mean and standard deviation values of physical characteristics of patient and control groups

	Patient	Control
Height	$170,08 \pm 13,2$	$169,09 \pm 11,1$
Weight	$119 \pm 21,11$	$63,15 \pm 10,9$
Body Mass Index	$40,72 \pm 6,8$	$22,16 \pm 1,97$

In the patient group (n=48), Ad-37 antibody positivity was detected in 39 samples, while in the control group (n=42), it was detected in 24 samples. According to the Mann Whitney U test result, a significant difference (p =0.011) was found in favor of the patient group.

For Adenovirus 5, antibody positivity was detected in 26 individuals in the patient group and 26 individuals in the control group. According to the Mann Whitney U test result, no statistically significant difference (p=0.461) was found.

More positivity for Adenovirus 37 was detected in the patient group compared to the control group. However, for Adenovirus 5, the number of positive individuals was equal in both patient and control groups. IgG positivity for both Ad-5 and Ad-37 was detected in 28 individuals in the patient group and 22 individuals in the control group. In 9 patient individuals who tested negative for Ad-37, Ad-5 was also negative. Ad-37 and Ad-5 were found to be more positive in male individuals in the patient group and female individuals in the control group. Comparison of Adenovirus 5 and 37 seropositivity by gender distribution in patient and control groups is shown in Table II.

Table II. Distribution of Ad-37 and Ad-5 antibody presence in patient and control groups by gender.

	Adenovirus 37 IgG	Adenovirus 37 IgG	Adenovirus 5 IgG	Adenovirus 5
	Positive	Negative	Positive	Negative
Patient (n=48) <i>Female (n=20) Male (n=28)</i>	39 14 25	9 6 3	26 10 16	22 10 12
Control (n=42) <i>Female (n=26) Male (n=16)</i>	24 16 8	18 10 8	26 15 11	16 11 5

There was no significant relationship found between the gender of individuals in patient and control groups and the positivity of Ad-5 and Ad-37 antibodies. The results of this relationship according to the Mann Whitney U test are shown in Table III.

Table III. Ad-37 and Ad-5 antibody positivity according to gender distribution in patient and control groups.

Control Group

	Gender	n	Rank Average	Rank Sum	U	P
Ad-5	Female	26	22,38	582,00	185,000	0,479
	Male	16	20,06	321,00		
Ad-37	Female	26	20,58	535,00	184,22	0,468
	Male	16	23,00	368,00		
Patient Group	Patient Group	Patient Group	Patient Group	Patient Group	Patient Group	Patient Group
Ad-5	Female	20	23,50	470,00	260,00	0,628
	Male	28	25,21	706,00		
Ad-37	Female	20	21,80	436,00	226,000	0,095
	Male	28	26,43	740,00		

DISCUSSION AND CONCLUSION

Obesity is defined as excessive weight gain resulting from the accumulation of fat in the body, and it is a rapidly spreading health problem worldwide. Factors such as increased consumption of processed foods, sedentary lifestyle, and processed food habits brought about by modern life are effective in the formation of obesity. Additionally, various factors such as nutritional and psychological disorders, endocrine system disorders, medication use, and genetic factors can contribute to obesity (2). In recent years, studies have been conducted on the concept of "infectobesity," suggesting that some microorganisms may also contribute

to obesity. According to this theory, certain infections can alter fat regulation in the body through various mechanisms and increase body mass index (4). This theory is supported by numerous pieces of evidence. Many reports investigating this issue have explained how infections caused by pathogens alter adipose tissue structure and support the role of infected adipocytes in obesity by disrupting components such as fat tissue signaling pathways (7).

Understanding these microorganisms as etiological factors is essential for effective management of obesity. Viral agents that are likely to play a role in fat regulation in the body have been found in human and animal studies conducted since 1982, including Canine Distemper Virus (CDV), Borna Disease Virus, Scrapie Agent, Rous-associated virus-7, SMAM-1, and Adenoviruses (8). Adenoviruses generally cause various infections in humans, primarily respiratory tract infections. Studies have shown that Ad-5, Ad-36, and Ad-37 may also cause obesity in humans. It is believed that these adenovirus serotypes have a negative effect on leptin production in infected adipocytes, leading to increased fat storage (9, 10).

The metabolic effects of adenoviruses have attracted more attention since it was shown in the 1990s that SMAM1 increases fat cell counts in chickens and that antibodies against SMAM-1 are associated with human obesity. Since then, a total of nine adenoviruses, including Ad-2, Ad-5, Ad-8, Ad-9, Ad-36, and Ad-37, have been investigated for their metabolic effects in cell cultures, animal studies, and human epidemiological studies. Among these, Ad-36 is the most studied serotype and has been most strongly associated with human obesity. While the association between Ad-2, Ad-8, and Ad-9 serotypes and fat accumulation in cells was not found in most studies, Ad-5 and Ad-37 have been identified as potentially related to obesity in different studies (11).

In a study investigating the effects of different adenovirus serotypes (Ad-36, Ad-2, Ad-9, and Ad-37) on leptin secretion and fat accumulation in 3T3-L1 preadipocyte cells, it was observed that leptin secretion in cells infected with Ad-36 was 50% lower than in uninfected cells, while lipid accumulation was significantly higher in cells infected with Ad-9, Ad-36, and Ad-37, with lower leptin secretion. Ad-2 was found not to affect lipid accumulation or leptin secretion (12). The decrease in leptin, also known as the appetite-suppressing hormone, is thought to be associated with weight gain at this level.

In our study, Ad-5 and Ad-37 serotypes were investigated in serum samples collected from obese patients and normal-weight control groups using ELISA kits. Ad-37 was found to be positive in 39 out of 48 obese patients and in 24 out of 42 individuals in the control group, while Ad-5 was positive in 26 individuals in both the patient and control groups. According to the statistical analysis results, a significant difference was found between the patient and control groups in terms of Ad-37 positivity, supporting our hypothesis. The positivity of Ad-37 serotype was interpreted as a possible contribution to the development of obesity. However, no significant difference was found for Ad-5, contrary to our hypothesis.

In a study by Whigham et al., chickens were inoculated with Adenovirus 2, 31, and 37, and food intake and weight were monitored for three and a half weeks. It was observed that chickens infected with Ad-37 had over three times the visceral fat and over twice the total body fat compared to the uninfected control group, despite consuming the same amount of feed (13).

In a study conducted on 268 patients with non-alcoholic fatty liver disease, the seropositivity of Ad-36 and Ad-37 was investigated. Ad-37 was positive in 65 patients, while both Ad-37 and Ad-36 were negative in 82 patients. The prevalence of obesity defined as a body mass index ≥ 30 did not differ significantly between patients positive for Ad-37 (11/65; 16.9%) and those negative for Ad-37 (15/82; 18.2%) (14).

In a two-stage study, antibodies for Ad-2, Ad-31, Ad-36, and Ad-37 were searched for in the serum of 502 obese and non-obese individuals, and no difference in antibody status was found between obese and non-obese individuals. In the second stage, the body mass index and body fat of twins discordant for Ad-36 antibody were compared between obese and normal-weight individuals, and it was found that the twin positive for Ad-36 antibody was heavier than their sibling. However, this relationship could not be established for Ad-2, Ad-31, and Ad-37 serotypes (15).

In the molecular-level investigation of epithelial cells infected with Ad-5, it was observed that Ad-5 has a metabolic effect similar to Ad-36 in triggering adiposity-related intracellular signaling mechanisms (11).

In a study by Cakmaklıoğlu et al. in 2011 investigating the seropositivity of Ad-5, Ad-8, and Ad-36 in obese and non-obese children, the positivity of Ad-36 and Ad-5 antibodies in the obese group was found to be statistically significant compared to the non-obese group. The study concluded that there was an association between Ad-5 and Ad-36 serotypes and obesity in children (8).

In a study by So et al., mice injected with Ad-5 showed significantly greater weight gain than the control group after 21 weeks. Additionally, there was no significant difference in food intake between the study and control groups, indicating that the weight difference was due to adenovirus infection (16).

In a study investigating the effect of Ad-5 on hamsters fed a balanced diet and a high-calorie diet, the experimental group was inoculated with Ad-5, and body weight was measured weekly for short-term (22 weeks) and long-term (44 weeks) periods. Adenovirus infection was shown to induce hyperglycemia and hyperlipidemia in animals fed a balanced diet. After 44 weeks, a 30% increase in body weight was reported compared to unvaccinated animals, along with morphological changes associated with non-alcoholic fatty liver disease. Similar but more severe changes were observed in animals fed a high-calorie diet, with the animals with the most morphological and functional changes having the lowest body weight (17).

Studies investigating the relationship between Ad-5 and Ad-37 and human obesity are limited worldwide. A definitive conclusion about whether these serotypes can cause obesity has not been reached. Our study found significant Ad-37 IgG positivity in obese individuals compared to the control group. This result will contribute to the literature by demonstrating the relationship between Ad-37 and obesity. However, Ad-5 IgG yielded insignificant results in both the obese and control groups. The presence of twenty-four Ad-37 positive individuals in the control group suggests previous exposure to this virus due to consumption under unhygienic conditions.

Further research on this topic in different geographical areas could provide clearer epidemiological information on viral obesity and infectobesity in general. As our knowledge of the relationship between microorganisms and obesity increases, the inclusion of antimicrobials in the treatment options for obesity seems likely in the near future.

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