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ORIGINAL ARTICLE

Adenovirus-5 and adenovirus-37 seropositivity in obese patients

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ABSTRACT

Background and Objectives: Obesity is a major health issue linked to conditions like diabetes, hypertension, and hyperlipidemia. Infectobesity suggests that certain microorganisms may contribute to obesity. Human adenovirus serotypes, particularly Human adenovirus type-36 (HAdV-36), Human adenovirus type-5 (HAdV-5), and Human adenovirus type-37 (HAdV-37), are thought to influence body fat regulation. This study investigates the relationship between Immunoglobulin G (IgG) positivity for HAdV-5 and HAdV-37 and obesity, aiming to provide data on the infectious etiology of obesity.
Materials and Methods: Blood samples separated into serums from obese (BMI ≥30) and non-obese (BMI 18.5-25) individuals were tested for HAdV-5 and HAdV-37 seropositivity using ELISA kits and seropositivity rates between the groups were compared.
Results: HAdV-37 antibody positivity was significantly higher in obese patients (39/48) compared to the control group (24/42) (p=0.011). For HAdV-5, antibody positivity was similar in both groups (26 individuals each) with no significant difference (p=0.461). No significant gender-related differences were found for either serotype.
Conclusion: The study suggests HAdV-37 may be associated with obesity, while no such relationship was found for HAdV-5. There was no gender association for either serotype. These results align with existing literature on HAdV-37, but further research is needed to confirm the link between adenoviruses and obesity and explore potential treatment options.

Keywords: Obesity; Human adenovirus; Serotype; Adipocytes; Infections

INTRODUCTION

Obesity is recognized as a major health crisis due to its association with chronic conditions like diabetes, hypertension, and hyperlipidemia (1). Since the 1980s, obesity has escalated into a worldwide epidemic, impacting nations regardless of their level of development. This phenomenon is attributed to various factors such as environmental influences, genetic predispositions, hormonal imbalances, dietary habits, and lifestyle choices (2). Despite numerous interventions, primarily targeting nutrition as a definitive solution remains elusive.

Given the alarming rise in obesity rates, researchers have considered the role of microorganisms in its onset (3). This has led to the development of the 'infecto-

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besity' theory, which posits that certain microorganisms can trigger obesity in animal models. Among these microorganisms are various animal viruses like Canine Distemper Virus (CDV), Borna Disease Virus (BDV), Rous-Associated Virus-7 (RAV-7), Scrapie prion, and Simitis Magdeburg Avian Malady-1 (SMAM-1), along with human viruses from the Adenoviridae family, such as Human Adenovirus 36 (HAdV-36), Human Adenovirus 37 (HAdV-37), and Human Adenovirus 5 (HAdV-5) (4).

Adenoviruses are medium-sized, non-enveloped viruses featuring an icosahedral shape and a genome made up of double-stranded DNA. There are over 50 different serotypes of these viruses capable of infecting humans (5).

Researches have shown that HAdV-5 and HAdV-37 promote fat cell formation in animal studies and are linked to obesity development. Among the human adenoviruses linked to obesity, HAdV-36 has received the most extensive research focus (6).

Leptin, a hormone secreted by fat cells, is crucial for regulating appetite, increasing energy expenditure, breaking down fat cells, and inhibiting lipid synthesis. Insufficient leptin production is associated with increased fat storage (7). To understand how adenovıruses affect fat tissue, studies have analyzed leptin secretion in human pre-adipocyte cells infected with these viruses. Results indicated that HAdV-37 infection led to reduced leptin levels and greater lipid accumulation, as well as changes in enzymes which play a role in the maturatıon of pre-adipocytes (3).

Our research aims to explore the connection between IgG positivity for HAdV-5 and HAdV-37 and obesity, thereby contributing to the understanding of the infectious causes of obesity.

MATERIALS AND METHODS

Ethical statement. This research took place at the İstanbul Aydın University Medical Microbiology Laboratory and received authorization from the İstanbul Aydın University Non-Interventional Clinical Research Ethics Committee under decision number 2023/16. Following our ethics committee approval, study groups were formed on February 8, 2023, and concluded on March 30, 2023. Consent was secured from all participants involved in the study.

Sample collection. The sample size was determined using the GPower 3.1.9.4 software, which calculated that 28 samples per group were needed to achieve a minimum statistical power of 0.90, with a type I error rate of 0.05 and an effect size of 0.5. The obese patient group consisted of patients applying to İstanbul Aydın University VM Medical Park Hospital for obesity surgery, while the normal weight control group was formed voluntarily from students and employees. Participants aged 18 to 50 who did not have any chronic illnesses or immune system disorders (based on the medical records of patients for obese individuals and according to the statements of participants for the healthy group) were selected. For obese patients, Body Mass Index (BMI) was ≥30, and for the healthy group, BMI was between 18.5 and 25. Blood samples were taken from individuals in fasting state who met these criteria, then centrifuged to separate the serums, which were subsequently stored at -20°C until analysis.

ELISA tests. To assess seropositivity for HAdV-37 and HAdV-5, we used ELISA test kits (SunRedBio - Republic of China) to identify antigen-antibody interactions via enzymatic reactions. Serum samples from both obese and healthy participants were examined following the protocol provided with the kits. Optical density readings were recorded at 450 nm using a Biotek-Epoch ELISA microplate reader.

Statistical analysis. The average value of the standard samples provided in the ELISA kits was used as the cut-off point to classify results as either positive or negative. The Shapiro-Wilk test was employed to assess data normality, revealing that the data did not follow a normal distribution. To analyze statistical significance and relationships between the results, non-parametric methods were applied, specifically the independent two-group Mann-Whitney U test, with a significance threshold set at $p < 0.05$.

RESULTS

Seropositivity analysis. In the group of patients, 39 samples tested positive for HAdV-37 antibodies, compared to 24 samples in the control group. The Mann-Whitney U test indicated a significant difference in favor of the patient group, with a p-value of 0.011.

Regarding Adenovirus 5, antibody positivity was

found in 26 individuals from both the patient and control groups. The Mann-Whitney U test showed no significant difference between these groups (p=0.461). The patient group showed a higher prevalence of positivity for Adenovirus 37 compared to the non-obese group. However, the rate of positivity for Adenovirus 5 was identical in both groups. In the patient cohort, 28 individuals tested positive for IgG antibodies against both HAdV-5 and HAdV-37, whereas only 22 individuals in the control group showed similar positivity. Among the 9 patients who were negative for HAdV-37, HAdV-5 was also absent. Table 1 provides a detailed comparison of HAdV-5 and HAdV-37 seropositivity by gender in both groups.

No significant association ($p<0.05$) was observed between the gender of individuals and the presence of HAdV-5 and HAdV-37 antibodies in both the patient and control groups.

Table 1. U test table for HAdV-37 and HAdV-5 seropositive patient and control groups according to gender variable

Patient Group	**Female (n:20)**		**Male (n:28)**		**P**
	n	**%**	**n**	**%**	
HAdV-5 IgG positive	10	50	25	89,2	0,628
HAdV-37 IgG positive	14	70	16	57,1	0,095
Control Group	**Female (n:26)**		**Male (n:16)**		**P**
	n	**%**	**n**	**%**	
HAdV-5 IgG positive	15	57,6	11	68,7	0,479
HAdV-37 IgG positive	16	61,5	8	50	0,468

DISCUSSION

Obesity is a health issue characterized by extreme fat accumulation in the body, and it is generally associated with various factors (2). According to 'infectobesity' theory, certain infections may impact body fat modulation, potentially resulting in an increased body mass index (BMI) (4). This theory is well-supported by considerable evidence, with numerous studies detailing how pathogen-induced infections can modify the structure of adipose tissue and disrupt components like fat signaling pathways, thereby highlighting the role of infected adipocytes in the development of obesity (8).

Effective management of obesity requires a comprehensive understanding of these microorganisms as etiological factors. Viral agents that have been implicated in regulating fat in the body in human and animal studies conducted since 1982 include CDV, BDV, Scrapie agent prion, RAV-7, SMAM-1, and Adenoviruses (9).

Adenoviruses generally lead to various infections in humans, with respiratory tract infections being the most frequent. Research has indicated that HAdV-5, HAdV-36, and HAdV-37 may also play a role in obesity. These particular adenovirus serotypes are believed to impair leptin production in infected fat cells, resulting in greater fat accumulation (10, 11).

Interest in the metabolic effects of adenoviruses grew substantially after the 1990s when it was discovered that SMAM-1 caused an increase in fat cells in chickens. Since that time, nine adenoviruses, including Human adenovirus 2 (HAdV-2), HAdV-5, Human adenovirus 8 (HAdV-8), Human adenovirus 9 (HAdV-9), HAdV-36, and HAdV-37, have been studied for their adipogenic effects in cell cultures and animal models. HAdV-36 has been the most thoroughly investigated and shows the strongest association with human obesity. Although many studies did not find a link between HAdV-2, HAdV-8, and HAdV-9 serotypes and lipid accumulation in cells, HAdV-5 and HAdV-37 have been implicated in obesity in various studies (12).

A study examining the impact of various serotypes (HAdV-36, HAdV-2, HAdV-9, and HAdV-37) on leptin secretion and lipid buildup in preadipocyte cells revealed that leptin levels in cells infected with HAdV-36 were cut by 50% compared to those not infected. Moreover, a marked increase in lipid accumulation and a decrease in leptin secretion were observed in cells infected with HAdV-9, HAdV-36, and HAdV-37. On the other hand, HAdV-2 did not influence either fat accumulation or leptin production. The notable reduction in leptin, a hormone that suppresses appetite, suggests a possible link to weight gain (13).

In our study, serum samples from both obese individuals and normal-weight controls were analyzed for HAdV-5 and HAdV-37 serotypes using ELISA kits. HAdV-37 positivity was detected in 39 of 48 obese patients and 24 of 42 control individuals, while HAdV-5 positivity was observed in 26 obese patients and 26 controls. Statistical analysis revealed a significant difference in HAdV-37 positivity between the obese and control groups, supporting our hy-

pothesis that HAdV-37 may be associated with obesity. However, there was no notable difference observed for HAdV-5, which was inconsistent with our hypothesis.

In a study, chickens inoculated with HAdV-2, Human adenoviruse 31 (HAdV-31), and HAdV-37 showed significantly higher percentages of visceral and total body fat were observed compared to uninfected control groups, even though both groups consumed the same diet (14). In a study on non-alcoholic fatty liver disease, researchers examined seropositivity for HAdV-36 and HAdV-37 and found no significant difference in obesity rates between individuals who tested positive and those who tested negative for HAdV-37 (15).

In a two-phase study, the first phase involved analyzing antibody levels for HAdV-2, HAdV-31, HAdV-36, and HAdV-37 in serum samples from obese and normal weight individuals, which revealed no differences between the two groups. In the second phase, twins with different HAdV-36 antibody statuses were compared, revealing that the twin with HAdV-36 antibodies was heavier. However, this weight difference was not seen with HAdV-2, HAdV-31, or HAdV-37 serotypes (16). Molecular-level research on epithelial cells infected with HAdV-5 has shown that this virus triggers metabolic changes akin to the intracellular signaling pathways employed by HAdV-36 to enhance fat accumulation (12).

In a study conducted by Çakmaklıoğulları et al., which examined HAdV-5, HAdV-8, and HAdV-36 seropositivity in obese children, it was found that the presence of antibodies against HAdV-36 and HAdV-5 was higher in the obese group compared to the normal weight group (9).

In a study mice that were injected with HAdV-5 showed a marked increase in weight gain after 21 weeks when compared to the control group. However, food intake did not significantly differ between the groups, indicating that the increase in weight was likely attributable to the adenovirus infection rather than changes in diet (17).

In a study evaluating the impact of Ad-5 on hamsters given either a standard diet or a high-calorie diet, the experimental group received Ad-5 inoculation. Body weight measurements were taken weekly for both short-term (22 weeks) and long-term (44 weeks) periods. The findings revealed that Ad-5 infection in hamsters on a standard diet resulted in elevated blood sugar levels and increased lipid levels. After 44 weeks, the group exposed to the virus showed a 30% rise in body weight compared to the non-inoculated group, along with morphological changes consistent with fatty liver disease. Animals fed a high-calorie diet showed similar but more severe changes (18).

Research on the potential role of HAdV-5 and HAdV-37 strains in the pathogenesis of obesity has been limited. No definitive conclusion has been reached regarding the ability of these serotypes to cause obesity. Therefore, there is a significant need for further research to deeply explore the relationship between HAdV-5 and HAdV-37 and obesity. Our study found a significant increase in HAdV-37 IgG among obese individuals compared to the control group, aligning with existing literature. The presence of 24 HAdV-37 positive individuals in the control group likely indicates prior exposure to the virus, possibly due to consuming contaminated food in unsanitary conditions.

Limitations of our study include measuring seropositivity for HAdV-5 and HAdV-37 at only one time point. Longitudinal data would be necessary to understand the persistence and fluctuation of antibody levels over time and their potential impact on obesity. Although the sample size was determined using GPower analysis, our study's relatively small sample size may limit the generalizability of the findings. Additionally, the study was conducted in a single geographic location, which may limit the applicability of the findings to other regions with different environmental, genetic, and cultural factors influencing obesity and viral exposure.

In conclusion, our study has shown a potential relationship between HAdV-37 and obesity. This finding may help us understand the role of viral infections in the pathogenesis of obesity. Further research involving different parameters is needed to explore the effects of HAdV-37 on obesity in more depth, which could contribute to the development of new strategies for the prevention and treatment of obesity. Therefore, this study is an important step in understanding and intervening in viral infections associated with obesity, making a significant contribution to the literature. Additional research across various geographical regions could offer more comprehensive epidemiological insights into viral obesity and infectobesity as a whole.

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The study protocol has been approved by the İstanbul Aydın University's Non-Interventional Clinical Research Ethics Committee with decision number 2023/16 (Reference number= E-18457941-050.99-54373). The study was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its following updates. This study was carried out and adapted from the PhD thesis work of Miss Nur Gamze Bostan.

REFERENCES

1. Shang Q, Wang H, Song Y, Wei L, Lavebratt C, Zhang F, et al. Serological data analyses show that adenovirus 36 infection is associated with obesity: A meta-analysis involving 5739 subjects. *Obesity (Silver Spring)* 2014; 22: 895-900.
2. Katzmarzyk PT, Chaput JP, Fogelholm M, Hu G, Maher C, Maia J, et al. International study of childhood obesity, lifestyle and the environment (ISCOLE): contributions to understanding the global obesity epidemic. *Nutrients* 2019; 11: 848.
3. Dhurandhar NV. Infectobesity: obesity of infectious origin. *J Nutr* 2001; 131: 2794S-2797S.
4. Na H-N, Nam J-H. Infectobesity: a new area for microbiological and virological research. *J Bacteriol Virol* 2011; 41: 65-76.
5. Ponterio E, Gnessi L. Adenovirus 36 and obesity: An overview. *Viruses* 2015; 7: 3719-3740.
6. Atkinson RL. Viruses as an etiology of obesity. *Mayo Clin Proc* 2007; 82: 1192-1198.
7. Paracchını V, Pedotti P, Taioli E. Genetics of leptin and obesity: A HuGE review. *Am J Epidemiol* 2005; 162: 101-114.
8. López-Ortega O, Moreno-Corona NC, Cruz-Holguin VJ, Garcia-Gonzalez LD, Helguera- Repetto AC, Romero-Valdovinos M, et al. The immune response in adipocytes and Their susceptibility to infection: A possible relationship with infectobesity. *Int J Mol Sci* 2022; 23: 6154.
9. Cakmakliogullari EK, Sanlidag T, Ersoy B, Akcali S, Var A, Cicek C. Are human adenovirus-5 and 36 associated with obesity in children? *J Investig Med* 2014; 62: 821-824.
10. Sapunar J, Fonseca L, Molina V, Ortiz E, Barra MI, Reimer C, et al. Adenovirus 36 seropositivity is related to obesity risk, glycemic control, and leptin levels in Chilean subjects. *Int J Obes (Lond)* 2020; 44: 159-166.
11. Karamese M, Altoparlak U, Turgut A, Aydogdu S, Karamese SA. The relationship between adenovirus-36 seropositivity, obesity and metabolic profile in Turkish children and adults. *Epidemiol Infect* 2015; 143: 3550-3556.
12. Voss JD, Atkinson RL, Dhurandhar NV. Role of adenoviruses in obesity. *Rev Med Virol* 2015; 25: 379-387.
13. Vangipuram SD, Yu M, Tian J, Stanhope KL, Pasarica M, Havel PJ, et al. Adipogenic human adenovirus-36 reduces leptin expression and secretion and increases glucose uptake by fat cells. *Int J Obes (Lond)* 2007; 31: 87-96.
14. Whigham LD, Israel BA, Atkinson RL. Adipogenic potential of multiple human adenoviruses *in vivo* and *in vitro* in animals. *Am J Physiol Regul Integr Comp Physiol 2*006; 290: R190-R194.
15. Trovato GM, Martines GF, Pirri C, Trovato FM, Castro A, Garozzo A, et al. Obesity-independent AAssociation of HHuman adenovirus Ad37 seropositivity with onalcoholic fatty liver disease. *J Clin Gastroenterol* 2012; 46(6): e46-54.
16. Atkinson RL, Dhurandhar NV, Allison DB, Bowen RL, Israel BA, Albu JB, et al. Human adenovirus-36 is associated with increased body weight and paradoxical reduction of serum lipids. *Int J Obes (Lond)* 2005; 29: 281-286.
17. So PW, Herlihy AH, Bell JD. Adiposity induced by adenovirus 5 inoculation. *Int J Obes (Lond)* 2005; 29: 603-606.
18. Montes-Galindo DA, Espiritu-Mojarro AC, Melnikov V, Moy-López NA, Soriano-Hernandez AD, Galvan-Salazar HR, et al. Adenovirus 5 produces obesity and adverse metabolic, morphological, and functional changes in the long term in animals fed a balanced diet or a high-fat diet: a study on hamsters. *Arch Virol* 2019; 164: 775-786.