

Association of human adenovirus-36 with diabetes, adiposity, and dyslipidaemia in Hong Kong Chinese

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KEY MESSAGES

1. Adenovirus-36 (Ad-36) is the only human adenovirus currently known to be associated with human obesity.
2. The Ad-36 antibody and Ad-36 DNA in both the plasma and stool of four groups of subjects stratified by diabetes and obesity status were examined.
3. Although the detection rate of Ad-36 plasma DNA was similar among the four groups, participants with obesity or diabetes had a higher rate of Ad-36 infection. In addition, participants with Ad-36 infection had lower high-density lipoprotein (HDL) than those without.

Nonetheless, the lower HDL in Ad-36 positive subjects may be confounded by age, obesity and diabetes status.

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Introduction

Obesity is a major health concern.¹ The first report of obesity caused by a virus was published in 1982,² and four other animal viruses have been identified since. Of the 51 human adenoviruses, three have been shown to cause obesity in animal models, whereas two have not.^{3,4} This study aimed to examine the association of human adenovirus-36 (Ad-36) with diabetes, adiposity, and dyslipidaemia in Hong Kong Chinese.

Methods

Ethical approval was obtained from the CUHK Clinical Research Ethics Committee. The body mass index (BMI) cut-off value was defined as 23 kg/m² for overweight and 25 kg/m² for obesity. This study was conducted from April 2009 to March 2011. A total of 303 serum samples were collected from four groups of local Chinese subjects aged 25 to 55 years with no family history of diabetes: (1) non-obese (BMI <23 kg/m²) non-diabetic controls (n=72), (2) obese (BMI ≥25 kg/m²) non-diabetic subjects (n=93), (3) obese diabetic subjects (n=70), and (4) non-obese diabetic subjects (n=68).

Results

Adenovirus-36 DNA in serum samples

A pair of primers was designed to study Ad-36. The specificity of the primers was evaluated with

adenoviruses serotype 1 to 6. A few unspecific bands in the other serotypes were observed with the naked eye, but they were very low in intensity and did not significantly affect the analyses (Fig). All 303 serum samples were screened with PCR assay using set B primer.

The Ad-36 DNA band size 149bp was amplified with set B primer. Among the 4 groups, non-obese diabetic subjects had the highest positive rate, followed by obese non-diabetic subjects, obese diabetic subjects, and non-obese non-diabetic subjects. The positive rate for the obese and non-obese groups was 7.4% and 7.1%, respectively (P=0.5608, Table 1).

Isolation of Ad-36 DNA from serum was technically challenging. Although positive controls using Ad-36 DNA as a template gave a DNA sequence that matched 100% with the expected published sequence, direct sequencing of the PCR from serum samples did not generate good sequence information, probably owing to an extremely low yield of PCR products. PCR with the primer set C yielded no bands with the serum DNA samples.

Antibody neutralisation tests

Antibodies in the sera were assayed by antibody neutralisation tests using Ad-36 grown in A549 cells. Each test serum was run in duplicate with a serum control (serum and cells, no virus), cell control (cells only, no serum, no virus) and virus control (cells and virus, no serum). Serum samples were considered

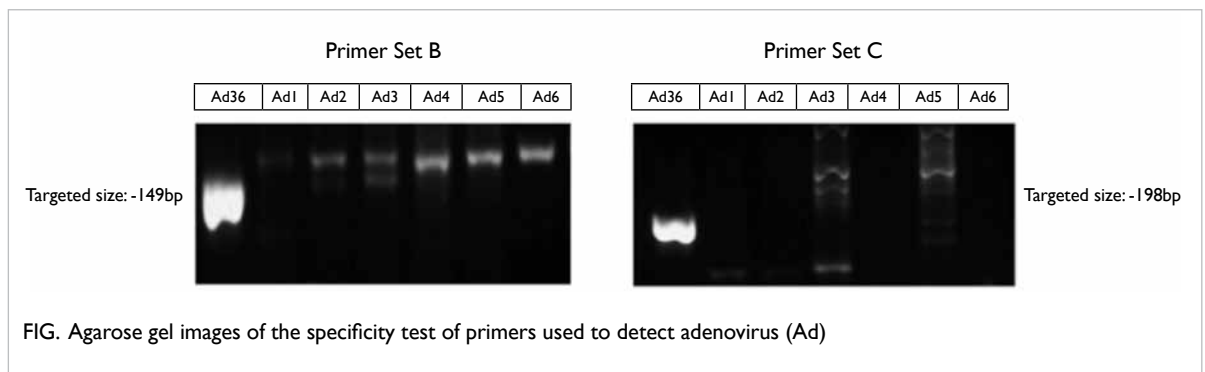


TABLE 1. Adenovirus-36 DNA positive rates for the four groups stratified by diabetes and obesity status and the overall obese and non-obese groups

Group	No. (%) of subjects			
	Strong band	Weak band	No band	Positive rate
Non-obese non-diabetic (controls, n=72)	1 (1.39)	2 (2.78)	69 (95.83)	3 (4.1)
Obese non-diabetic (n=93)	3 (3.23)	5 (5.38)	85 (91.40)	8 (8.6)
Obese diabetic (n=70)	2 (2.86)	2 (2.86)	66 (94.29)	4 (5.7)
Non-obese diabetic (n=68)	2 (2.94)	5 (7.35)	61 (89.71)	7 (10.3)
Overall*				
Obese (n=163)	5 (6.09)	7 (8.24)	151 (92.6)	12 (7.4)
Non-obese (n=140)	3 (4.33)	7 (10.13)	130 (92.9)	10 (7.1)

* P=0.5608, Fisher's exact test one-tailed probability

TABLE 2. Antibody neutralisation test for adenovirus-36

Variable	Total (n=174)	Ad-36 positive (n=30)	Ad-36 negative (n=144)	P value (t test or Chi squared test)
Mean±SD age (years)	45.9±7.3	49.6±4.4	45.2±7.5	0.002
No. (%) of females	95 (54.6)	17 (56.7)	78 (54.2)	0.843
No. (%) of obesity	80 (46.0)	18 (60.0)	62 (43.1)	0.090
No. (%) of diabetes	132 (75.9)	28 (93.3)	104 (72.2)	0.014

TABLE 3. Stool DNA PCR results

Group	No. (%) of adenovirus-36 DNA in stool	P value
Obese (n=100)	11 (11)	0.34
Non-obese (n=67)	11 (16)	
Diabetic (n=63)	12 (19)	0.132
Non-diabetic (n=104)	12 (12)	
Non-obese non-diabetic (n=53)	6 (11)	0.132
Non-obese diabetic (n=14)	5 (35)	
Obese non-diabetic (n=51)	4 (7)	0.24
Obese diabetic (n=49)	7 (14)	

to contain neutralising antibodies when 50% of the wells showed a cytopathic effect. Obese subjects had a higher percentage of antibody-positive samples than non-obese subjects (Table 2).

Stool DNA analyses

Stool DNA was also studied by PCR using primer set B. The positivity rate of Ad-36 DNA in the stool was higher in non-obese diabetic subjects than non-obese non-diabetic subjects (35% vs 11%, P=0.042). Stool samples were insufficient to use the nanodrop PCR method to amplify the DNA, thus many stool samples had an undetectable level of DNA. The results of the Ad-36 DNA study in stool samples using primer set B are shown in Table 3.

Discussion

Of 278 participants, 174 had valid measures of lipid profile. Participants with Ad-36 infection were older and more likely to have diabetes, compared with participants without Ad-36 infection. In addition, there was a marginal difference in obesity between participants with and without Ad-36 infection. Participants with Ad-36 infection had significantly

lower high-density lipoprotein (HDL) than those without (1.30 ± 0.37 mmol/L vs 1.49 ± 0.44 mmol/L), although this difference was not significant after adjusting for confounding factors (age, sex, obesity, and diabetes). No significant difference was noted in total cholesterol, triglycerides, or low-density lipoprotein in either univariate analyses or multivariate analyses (data not shown).

The rate of Ad-36 DNA in stool samples (antibody positive rate) was higher in non-obese diabetic subjects than non-obese non-diabetic subjects (35% vs 11%, $P=0.042$). Two possible reasons for the slightly higher rate in our group compared with other studies are: (1) the cut-off point to define our obese subjects was lower, as we applied the Asian definition of obesity rather than that of the World Health Organization. Thus, the categorisation of BMI in obesity classes based on co-morbidity risk might not operate for adiposity related to adenovirus infection. (2) Microbes other than Ad-36 virus might be more relevant in Hong Kong with regard to their role in the pathogenesis of obesity. In support of this notion, we incidentally detected a higher rate of a 200 bp band in the obese group, compared with the non-obese group. Upon cloning and sequence analyses, it is suggested that another microbe might be associated with obesity. The identity of the microbe is not certain but our preliminary results indicate that its DNA sequence matches that of *Babesia gibsoni* 16S ribosomal RNA (data not shown).

Conclusions

Obese or diabetic subjects had a higher rate of Ad-36

infection. Those with Ad-36 infection also had lower HDL than those without, but this finding may have been confounded by age, obesity and diabetes status. Non-obese diabetic subjects had a higher rate of Ad-36 DNA in the stool than non-obese non-diabetic subjects. These findings support the possible role of viral or microbial infection in both obesity and diabetes, although larger cohort studies together with mechanistic studies are needed to confirm these findings.

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