

## Carotid Intima-Media Thickness in visceral obese Egyptian female adolescents with adenovirus 36 infection

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## Abstract

**Background:** Increased carotid intima- media thickness (CIMT), a marker of early- onset atherosclerosis, has been observed in obese adolescents. Recently the viral infection increased interest in obesity- related studies, especially adenovirus 36.

**Aim:** To investigate the relationship between CIMT, visceral obesity and adenovirus 36 infection in female adolescents. Study Design: A cross-sectional study included 90 females aged (12- 15) years. Anthropometric assessment was done. Visceral obesity was measured by abdominal ultrasound. CIMT for both carotid arteries were measured by high- resolution echo- Doppler. Qualitative Human adenovirus 36 antibody was assessed using ELISA.

**Results:** Girls with visceral obesity had higher frequency of increased CIMT at left (96.2% versus 75%) and right carotid artery (84.6% versus 73.4%), and adenovirus 36 sero- positive antibodies than among those without visceral obesity  $p < 0.01$ . Visceral obesity; among total sample; had significant positive correlations with BMI, waist and hip circumference. While it had insignificant correlations with age, CIMT at both right and left carotid arteries and adenovirus 36.

**Conclusions:** The frequency of increased CIMT at left carotid artery was higher among girls with visceral obesity than among those without visceral obesity. Visceral obesity, CIMT at right& left carotid arteries, and Adenovirus- 36 had insignificant correlations with both.

**Keywords:** Carotid Intima- Media Thickness- Visceral Obesity- Adenovirus 36.

## سمك البطانة الداخلية للشريان السباتي في المراهقات المصابات بسمنه الأحشاء الداخليه مع وجود العدوى الفيروسيه لفيروس الغدى ٣٦

**المقدمه:** ان الزيادة في سمك البطانة الداخليه للشريان السباتي احدى المؤشرات لحدوث المبكر لتصلب الشريان في المراهقات البنءاء. حديثا زادت الدراسات التي لها علاقة بالبءاءة حول الفيروس الغدى ٣٦.

**الهدف:** دراسته العلاقة بين سمك البطانة للشريان السباتي وسمنه الأحشاء الداخليه وعدوى فيروس الغدى ٣٦.

**تصميم الدراسة:** دراسته مكونه من ٩٠ مراهقه من سن (١٢- ١٥) سنه. القياسات الانثرومومترية. تم عمل سونار للبطن وموجات فوق صوتيه على الرقيه وتحليل الاجسام المضاده للفيروس الغدى ٣٦.

**النتائج:** البنات ذات زياده في سمنه الأحشاء مع زياده في سمك البطانة الداخليه للشريان السباتي على الناحية الشمال واليمين. وجود اجسام مضاده للفيروس الغدى كانت اكثر نسبة مع البنات البنءاء مع زياده سمنه الأحشاء بينما لا توجد علاقة مع كتله الجسم ومحيط الخصر ومحيط الارءاف. ولا توجد علاقة قويه مع السن وسمك البطانة للشريان السباتي على الناحيتين اليمين والسماال والفيروس الغدى ٣٦.

**الاستخلص:** زياده سمك بطانته الشريان السباتي على الناحية الشمال مع المراهقات ذوات زياده سمنه الأحشاء اكثر من المراهقات بدون هذه الزياده.

سمنه الأحشاء وسمك البطانة للشريان السباتي على اليمين والسماال والفيروس الغدى ٣٦ ترابط غير مؤثر.

**الكلمات الداله:** سمك بطانته الشريان السباتي- سمنه الأحشاء- الفيروس الغدى ٣٦.

## Introduction:

Obesity is one of the most serious public health problems of the 21st century, and its prevalence is influenced by a complex association of genetic, behavioral and socio-economic factors. The remarkable dramatic increase in global prevalence of pediatric obesity, led to the growing of appearance of obesity-related co-morbidities at adolescence.<sup>(1)</sup>

Visceral fat accumulation causes a lot of metabolic disorders like insulin resistance and hypertension; through decreasing level of adipocytokines which leads to cardiovascular risks and premature death due to early atherosclerosis.<sup>(2)</sup> However, subclinical atherosclerosis starts in early childhood, then progress into the adult life.<sup>(3)</sup>

Increased carotid intima-media thickness (CIMT) and aortic stiffness; as markers of arterial atherosclerosis; have been greatly related (in both children and adults) to the occurrence of cardiovascular risk factor "CRF".<sup>(4)</sup> CIMT is a mirror reflecting an image of remodeling and smooth muscle cell hypertrophy due to increased blood pressures; one the most important cardiovascular risk factor.<sup>(5)</sup>

The aim of the current study was to investigate the relationship between visceral obesity, carotid intima-media thickness (CIMT) and adenovirus 36 among Egyptian adolescent females.

## Subjects And Methods

### Subjects:

This cross sectional study included 90 adolescent females; with age range (12- 15) years. It was conducted at the "Medical Excellence Research Center (MERC)" of the "National Research Centre", during the period between September 2016 and November 2017.

Ethical approvals were obtained from both the Ethical Committee of Faculty of Postgraduate Childhood Studies and from the National Research Centre (Approval No. 15089). A verbal approval was taken from every girl participated in the current study, in addition to a written informed consent from one of her parents, after explanation of the aim of the study, as well as its possible benefits in avoiding the hazardous health effects of obesity.

### Methods:

Every girl included in the study was evaluated by the following methods, a full history taking, thorough clinical general and local examination, anthropometric assessment, abdominal ultrasound, carotid artery ultrasonography and immunological assessment of adenovirus 36.

A full history was taken from apparently healthy participants. It included present (any present disease), past and family history (history of obesity, previous infection, hypertension, cardiovascular diseases and diabetes).

Thorough clinical general and local examination was done to exclude organic or genetic disorders; that might interfere with the subject's normal growth.

⌘ Anthropometric measurements: for every participant girl, the following anthropometric measurements were taken: body weight (Wt), height (Ht), waist (WC) and hip (HC) circumferences. Then,

BMI was calculated. All measurements were taken by a well-trained researcher and her assistant to him, using standardized equipments and following the recommendations of International Biological Program (I.B.P), and then the mean of three consecutive measurements was recorded.<sup>(6)</sup>

Body height was measured to the nearest 0.1 cm using a Holtain portable anthropometer (The Harpenden Portable Stadiometer, Wales, UK). Body weight was determined to the nearest 0.01 kg using Seca scale (Seca Balance Beam Scale Model 700, Seca Deutschland Medical Scales and Measuring Systems, Seca gmbh& co. kg. Hamburg, Germany), with the subject dressed minimum clothes and no shoes. Body mass index (BMI) was calculated as weight (in kilograms) divided by height (in meters) squared. Waist circumference was measured at the level of the umbilicus with the girl standing and breathing normally. Hip circumference was measured, while the participant girl was wearing light clothing; at the widest level over the greater trochanters in a standing position and by the same examiner. Circumferences were measured, using non-stretchable plastic tape, to the nearest 0.1 cm.

⌘ Abdominal ultrasonography: Ultrasound (US) examination, to every girl, was done to evaluate visceral fat at the umbilicus (USVF) in cm. Intra abdominal fat thickness measurement was obtained using the "Madison Sonoace X8" Ultrasonographic equipment. For the visceral fat, a 3.5 MHz transducer was transversely positioned 1 cm above the umbilical scar on the abdominal midline, without exerting any pressure over the abdomen. The visceral fat thickness attempted corresponding to the measurement in centimeters between the internal surface of the abdominal rectus muscle and the posterior aortic wall in the abdominal midline, during expiration. Subcutaneous fat(s) distance from the skin to the Linea Alba, measured on the hemisterna line, 1cm above the umbilical scar, utilizing the linear transducer in a longitudinal section. Umbilical fat normal value is 4.47cm, and girls with higher values were considered to have visceral obesity.<sup>(7)</sup>

⌘ Carotid artery ultrasonography: This examination was performed using a high-resolution echo-Doppler device with a 7 MHz linear transducer. All participants in the study were examined in the supine position, with the head overextended and turned 45° away from the examined side. Both carotid arteries were visualized longitudinally, so that the CIMT of their distal wall was apparent. The best images of the distal wall were used to calculate the CIMT of the common and internal carotid arteries. The value of the CIMT was defined as the mean value of measurements between the right and left carotid arteries, calculated from 10 measurements on each side, 10mm from the bifurcation of the common carotids.<sup>(8)</sup>

1. Cut off point for right common carotid artery= 0.39- 0.43 (25<sup>th</sup>- 75<sup>th</sup> percentile).

2. Cut off point for left common carotid artery= 0.30- 0.47 (25<sup>th</sup>- 75<sup>th</sup>

percentile).<sup>(9)</sup>

✧ Immunological analysis: Qualitative Human adenovirus 36 antibody (anti- ADV36) ELISA:

1. Principle of the assay: The kit uses a sandwich enzyme- linked immunosorbent assay (ELISA) to qualitatively analyze Human Adenovirus 36 Antibody (Anti- ADV36) in Human serum.
2. Determine The Result:
  - ✧ The average of the Positive control well is typically  $\geq 1.0$ ; the average of the Negative control well is typically  $\leq 0.15$ .
  - ✧ Actual OD values of the Positive and Negative control wells may vary depending on the assay conditions.
  - ✧ Cut- off values were the average of the Negative control well+ 0.15 in samples tested by the laboratory.

**Statistical Analysis:**

It was performed using the computer program SPSS statistical package software for Windows version 16 (SSPS Inc, Chicago, USA). Visceral fat; at umbilicus; cut off point of 4.47 cm was used to classify the girls under study into 2 groups: Those above 4.47 cm were considered to have visceral obesity, while those with  $\leq 4.47$  cm were considered without visceral obesity. Descriptive statistics (mean $\pm$  SD) were calculated for the anthropometric and the ultrasound findings. Student’s t- test was used to compare the 2 groups. Pearson’s correlation was used to assess the association between the carotid intima- media thickness and the anthropometric measurements. Standards of probability were set to  $P < 0.01$ ; which considered highly significant; and  $P < 0.05$ ; which is considered statistically significant; in all analyses.

**Results:**

Girls with visceral obesity had the highest significant values for he anthropometric parameters (Weight, BMI, WC, HIP C) and visceral fat at umbilicus ( $P < 0.05$ ). There were insignificant differences between the 2 groups in CIMT at right and left carotid Table (1).

Frequency distribution of increased CIMT at the right and left carotid arteries was presented in Fig. (1). The increase of CIMT at left carotid artery was more prominent than in right one in both groups.

Frequency distribution of the adenovirus among the girls without and with visceral obesity was present in figure (2). Adenovirus was detected among 36/ 64 girls without visceral obesity (56.2%), 18/ 26 girls with visceral obesity t (69.2%) with significant differences between the 2groups using Chi- square test ( $P < 0.05$ ).

There were insignificant negative correlations between the visceral obesity and CIMT at both right and left carotid arteries. CIMT at left carotid artery had highly significant positive correlation with CIMT at right carotid artery and insignificant correlations with the visceral obesity at umbilicus. Adenovirus 36 had insignificant correlations with visceral obesity, and CIMT at both right and left carotid arteries Table (2).

**Discussion:**

Measurement of Carotid intima- media thickness (CIMT), which is a non- invasive, feasible, reliable and inexpensive tool to diagnose early

vascular damage, is considered as a marker for increased cardiovascular risk and development of subclinical atherosclerosis in adults, as well as in children.<sup>(10)</sup>

Adenoviruses are considered one of the most popular viral infections in early childhood; responsible for nearly one third of upper respiratory tract infections; which leads to mild and mostly self- limiting diseases.<sup>(11)</sup> Adv- 36 is the only one of the human adenoviruses which was found to have correlation with the obesity in human being.<sup>(12)</sup>

In the current study, girls with visceral obesity had the highest significant values in BMI, WC, HIP C and visceral fat at umbilicus, compared to those without visceral obesity. This agrees with the studies of Al- Hazzaa et.al.<sup>(13)</sup> in Saudi Arabia; Kankana et.al.<sup>(14)</sup> in India; who found that adolescent females with visceral obesity had higher BMI, hip C and wide WC.

The current study revealed that CIMT at left carotid artery had significant positive correlation with CIMT at right carotid artery. It had insignificant correlations with the others anthropometric measurements, visceral obesity at umbilicus. The present study showed that frequency of adenovirus 36 was significantly higher among girls with visceral obesity (69.2%) than among girls without visceral obesity (56.2%). Our results agree with many studies, for example, Parra- Rojas et.al.<sup>(15)</sup> in Mexico and Cakmakliogullari et.al.<sup>(16)</sup> in Turkey. They found significantly higher frequency of Ad- 36 seropositive antibody among obese adolescents than normal peers. In united state study, Broderick et.al.<sup>(17)</sup> studied US military personnel, they found significant association between Adv 36 positivity and female gender; and attributed this to estrogens that may play an important role in increasing the susceptibility to Adv36 Infection. Moreover, Atkinson et.al.<sup>(18)</sup> and Gabbert et.al.<sup>(19)</sup> found a significantly greater waist circumference; as marker of visceral obesity; in Adv 36- positive obese Italian children, although Na, et.al.<sup>(20)</sup> did not find the same relation.

**Conclusions:**

The frequency of increased CIMT at left carotid artery was higher among girls with visceral obesity than among those without visceral obesity. However, visceral obesity, CIMT at both right and left carotid arteries, and Adenovirus- 36 had insignificant correlations with both.

Table (1) Characteristics of the girls with and without visceral obesity (Student’s t test)

Variable	Girls Without V Obesity (N= 64)		Girls With V Obesity (N= 26)		t	P	
	mean	+ Sd	mean	+ Sd			
Age (Years)	13.027	1.5448	13.683	1.283	- 1.912	0.059	
Anthropometry	Wt (Kg)	62.35	18.02	73.45	20.81	- 2.531	0.022
	Ht(Cm)	152.06	9.37	154.19	9.08	- 0.986	0.323
	BMI (Kg/m <sup>2</sup> )	26.21	6.14	30.47	6.78	- 2.888	0.008
	Wc (Cm)	80.30	12.98	91.69	10.94	- 3.940	0.000
	Hip(Cm)	98.33	16.55	107.08	15.94	- 2.297	0.024
Radiology	Visceral fat at umbilicus (cm)	3.02	0.60	5.32	0.87	- 12.328	0.000
	Rt Cimt (Mm)	0.05	0.01	0.05	0.01	- 0.164	0.854
	Lt Cimt (Mm)	0.05	0.01	0.05	0.00	- 0.430	0.735

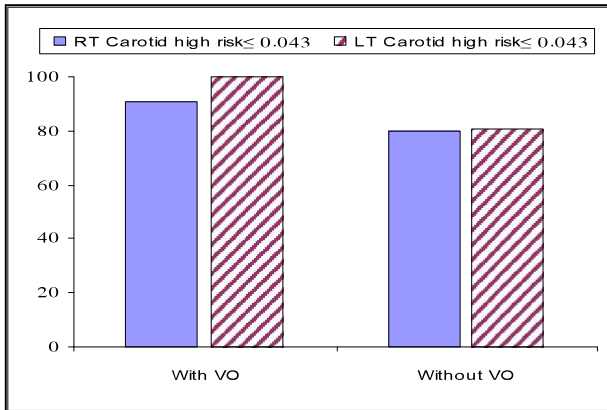


Figure (1) Frequency distribution for the high risk of right and left CIMT measurements among girls with and without visceral obesity

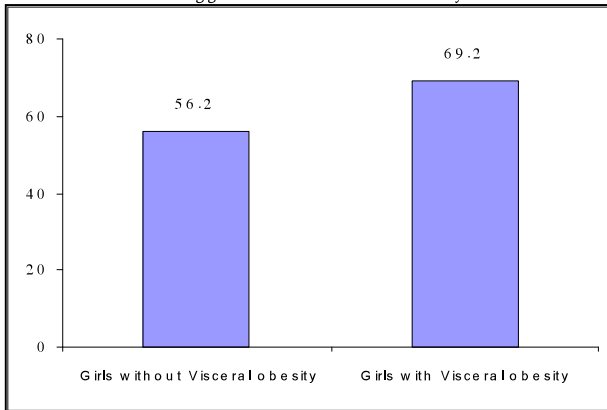


Figure (2) Frequency distribution of the adenovirus regarding visceral obesity  
Table (2) Correlation between the visceral obesity at umbilicus, adenovirus 36 and CIMT at left carotid artery with the radiological measurements among total sample

Variable	V Obesity		ADENO		Left CIMT	
	r	p	r	p	r	p
Visceral fat at umbilicus (cm)			0.081	0.451	-0.067	0.527
Rt Carotid (Mm)	-0.075	0.485	0.029	0.789	0.937**	0.000
Lt Carotid (Mm)	-0.067	0.527	0.059	0.583		

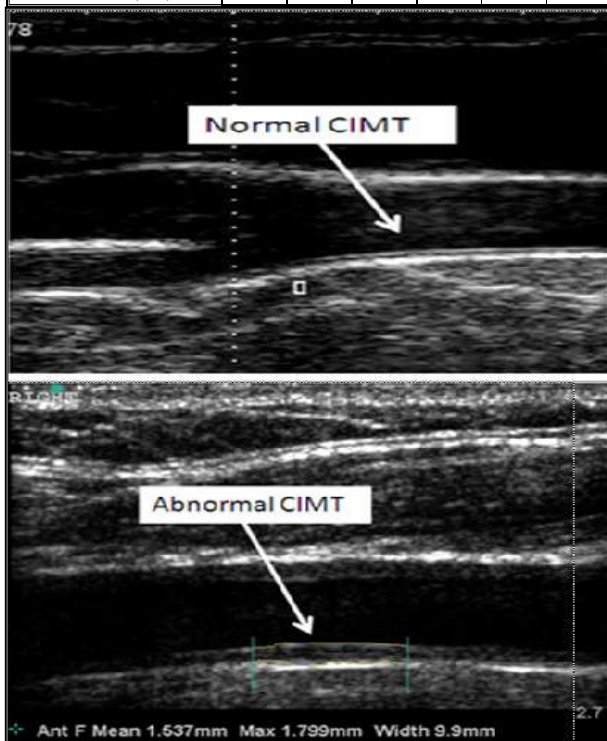


Figure (3) Comparison between normal and abnormal CIMT (Leite et.al., 2012).

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