The Possible Association of Adenovirus – 36 Infection with Obesity, DM – I and DM – II in some Groups of Iraqi Patients

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Abstract :
Obesity, DM-I, and DM-II are considered major health problems and life threats for some groups of people. Many studies have revealed that the prevalence of Adv36 infection in 30% or more in the obese adult population and dramatically human adenovirus-36 (Adv36) upregulates distal insulin signaling in vitro and in vivo studies. The current study aimed to explore the association of Adenovirus – 36 infections with obesity, DM-I, and DM-II in some groups of Iraqi patients, and according to our knowledge, in Iraq, there is no published study on this topic. In total, 90 serum samples of three age groups of patients were distributed as 45 samples of patients with DM-I and another 45 samples of patients with DM-II. The presence of Adv36 Abs in serum was determined by using the "Qualitative Human Adenovirus 36 Antibody (ADV36Ab) ELISA Kit". The prevalence of Adv36 positivity was higher in DM-II as (31.1%) followed by DM-I which estimated as (21.1%) in significant differences compared with the negativity ($p< 0.05$). Furthermore, that positivity was paralleled with the increase of obesity prevalence as (12.2%) in obese class I patients had been with DM-II ($p< 0.05$). In addition, the Adv36-positive serology test was more associated with (8 – 25) of age interval and patients with obese class I ($p< 0.05$), even more in females compared to males with significant differences ($p< 0.05$). The current results showed an association of Adv-36 antibodies existence with obesity and particularly it has been associated with overweight. Furthermore, Adv-36 positivity was related to DM-II patients first then followed by DM-1 for the Iraqi patients. Finally, our results give the warning to evaluate the issues with more highly efficient laboratories and clinical techniques to confirm the real role of Adv-36 as a co-factor with obesity in obese and diabetic Iraqi patients.
Keywords: Diabetes mellitus type -1 (DM-I), (DM-II), Human adenovirus-36, Obesity.

الارتباط المحتمل لعدوى الفيروس الغدي البشري –36 مع السمنة وداء السكري من النمط الأول والنمط الثاني لدى مجموعات مختارة من المرضى العراقيين

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الخلاصة :
تُعد السمنة وداء السكري من النمط الأول (DM–I) وداء السكري من النمط الثاني (DM–II) من أبرز المشكلات الصحية العامة وعوامل الخطورة الرئيسة المسببة للامراض والوفيات لمجموعة من الأشخاص . وقد أشارت عدة دراسات إلى أن معدل انتشار الإصابة بالفيروس الغدي البشري –36 (Adv36) قد يصل إلى 30% أو أكثر بين البالغين المصابين بالسمنة. كما بينت الدراسات التجريبية أن هذا الفيروس قادر على تعزيز مسارات الإشارة الخاصة بالإنسولين في الخلايا الحية *in vitro* و*in vivo*. هدفت الدراسة الحالية إلى استقصاء الارتباط المحتمل بين عدوى الفيروس الغدي –36 والسمنة وداء السكري بنوعيه (DM–I و DM–II) في مجموعات مختارة من المرضى العراقيين، حيث تُعد هذه الدراسة . بحسب معرفتنا . الأولى من نوعها في العراق.
تم جمع (90) عينة مصلية من ثلاث فئات عمرية لمرضى السكري، تضمنت (45) عينة لمرضى DM–I و(45) عينة لمرضى (DM–II) وجرى الكشف عن وجود الأجسام المضادة للفيروس الغدي –36 باستخدام عدة الفحص المناعي الإنزيمي النوعي (ELISA) الخاصة بالأجسام المضادة للفيروس الغدي 36 .(أظهرت النتائج أن نسبة الإيجابية المصلية للفيروس كانت أعلى لدى مرضى D ، مع وجود فروق معنوية عند المقارنة بالحالات السلبية.($p<0.05$) كما تبيّن أن

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الإيجابية المصلية ارتبطت بزيادة معدلات السمنة، خصوصاً بين مرضى DM–II من الفئة الأولى من السمنة (12.2 .(p<0.05) (%كذلك لوحظ أن إيجابية الفحص كانت أكثر شيوعاً في الفئة العمرية (8–25) سنة، وبين الإناث مقارنةً بالذكور بفروق معنوية.(p<0.05)
تشير النتائج الحالية إلى وجود ارتباط معنوي بين إيجابية الأجسام المضادة للفيروس الغدي –36 وكل من السمنة (خصوصاً زيادة الوزن والسمنة من الدرجة الأولى) و DM–IIثم DM–I لدى المرضى العراقيين. وتسلط هذه النتائج الضوء على الحاجة إلى إجراء دراسات واسعة النطاق باستخدام تقنيات مخبرية وإكلينيكية أكثر دقة لتوضيح الدور المحتمل للفيروس الغدي –36 كعامل مساعد في السمنة والسكري.
الكلمات المفتاحية :داء السكري من النمط الأول(DM–I) ، داء السكري من النمط الثاني(DM–II) ، الفيروس الغدي البشري –36، السمنة .

Introduction

Adenoviruses (members of Adenoviridae family), are naked viruses (without lipid bilayer envelope), viruses with an icosahedral nucleocapsid structure and ds-DNA viral core. Their name originates from their first site of isolation in human adenoids in 1953 [1,2].They have a wide spectrum of vertebrate hosts; in humans, more than 50 different serotypes of adenoviral interact with a broad sort of diseases[3,4]. Many previous studies revealed that mice or chickens inoculated with analogous kinds of viruses showed weight gain with statistically significant correlation [3-6]. On the basis of these studies it has been concluded a positive relationship between body fat formation and the existence of the Adv-36 Abs in the blood stream [3, 4]. There is a possibility and notifying evidence that obesity problems in humans are related to human adenovirus-36 (Adv36) infection. At the experimental level, animal's infection with Adv36 shows that this virus causes obesity. Many scattered reports explored the relationship of human and Adv36 infection have shown a prevalence of Adv36 infection in 30% or more in obese adult individual, but no conclusive proof of its association with obesity has yet been established ,more studies are required to identify prevalence and consequences of Adv36 infection in different age groups of people with different geographic locations [5, 6]. To date, the Adv-36 serotype is the only human adenovirus that has been linked obesity, which is present in 30% and 11% of obese and nonobese humans respectively [5, 6]. The adiposity progress by human adenovirus-36 (Adv36), but also experimentally can upregulated distal insulin signaling independently in vitro and in vivo of the rodent of adiposity. According to that, antibodies against Adv36 in children and healthy adults had reduced hepatic lipid accumulation and increased insulin sensitivity. Finally, I hypothesized that Adv36 infection would be also common in individuals with type-1 and type -2 diabetes [7, 8]. So that, the present study aimed to evaluate the potential association between Adenovirus-36 (Ad-36) infection and the prevalence of obesity, type 1 diabetes mellitus (DM-I), and type 2 diabetes mellitus (DM-II) among Iraqi patients. To the best of our knowledge, no prior research in Iraq has investigated this relationship.

Materials and methods Cases selection and study groups

This study involved (90) serum samples, distributed as forty five of (DM-I patients) as a first group of study , and another forty five cases of (DM-II) as a second study group, all of participants were attending the Specialist Centre for the Endocrine glands diseases and diabetes AL- Kindi hospital \ Baghdad, & a questionnaire contains information about each single patient included (Name , gender , age , smoking status ,BMI, History of DM-I & DM-II) was included, & the diagnosis of all cases for DM types is done by qualified physician and some other laboratory investigations based up on patients medical history. All samples were subjected to ELISA for Adv-36 antibodies detection. A local Ethical Committee reviewed and approved the study.

Methods ELISA Test of Human Adenovirus 36 Antibody (Adv36Ab) detection

This Kit uses technique of enzyme linked immunosorbent (ELISA) of double antigen sandwich way in order to analyze the presence or absence of Adv36 Ab in serum samples of human, all sera were tested for Adv36Ab according to the manufacture leaflet which was supplied from (MyBioSource company, Catalog .No: MBS9310682), by Adding sample to well pre – coated with Human adenovirus 36 antigen to combine the analyte , then incubation followed by procedures of washing to get rid of boundless

substance. Finally, the (HRP) substrate was added, followed by incubation for detection process, and the blue color was generated. The reaction ended, and color converted to yellow when added stopping solution (acidic). The presence or absence of Human (Adv36 Ab) in samples was evaluated by comparing the O.D. at 450 nm using a microtiter plate reader within 15 min of the samples to the CUT OFF [9].

Body Mass Index Measurement (BMI)

The (BMI) calculation is done by the following formula: BMI= weight (kg) / height2 (m2), for the (Metric Unit) , and classified according to the World Health Organization's (WHO) index into eight categories as shown in the table (1) below [10].

Table (1) : The Eight BMI categories \ WHO [10]*

Category	BMI range - kg/m^2
Severe Thinness	< 16
Moderate Thinness	16 - 17
Mild Thinness	17 - 18.5
Normal	18.5 - 25
Overweight	25 - 30
Obese Class I	30 - 35
Obese Class II	35 - 40
Obese Class III	> 40

Statistical analysis

Chi-square was used to detect significant differences among the variables of our study. By using SPSS ver. 18.0.

Results

BMI Classification for the study groups

The seven categories of BMI for diabetic patients were showed and classified according to WHO scales in figure (1) , as 28 (31.1%) over weight patients (BMI range 25–30 kg/m2), 12 (13.3%) patients were Obese Class III (BMI range > 40 kg/m2), 6 (6.7%) subjects with Obese Class II (BMI 35 -40 kg/m2) and 17 (18.9%) subjects (BMI 30 -35 kg/m2) as Obese Class I, 8 (8.9%) patients were with normal (BMI 18.5 -25 kg/m2),10 (11.1%) of patients were with moderate thinness (BMI 16 -17 kg/m2) & only 9 (10%) patients were as mild thinness (BMI 17 -18.5 kg/m2).

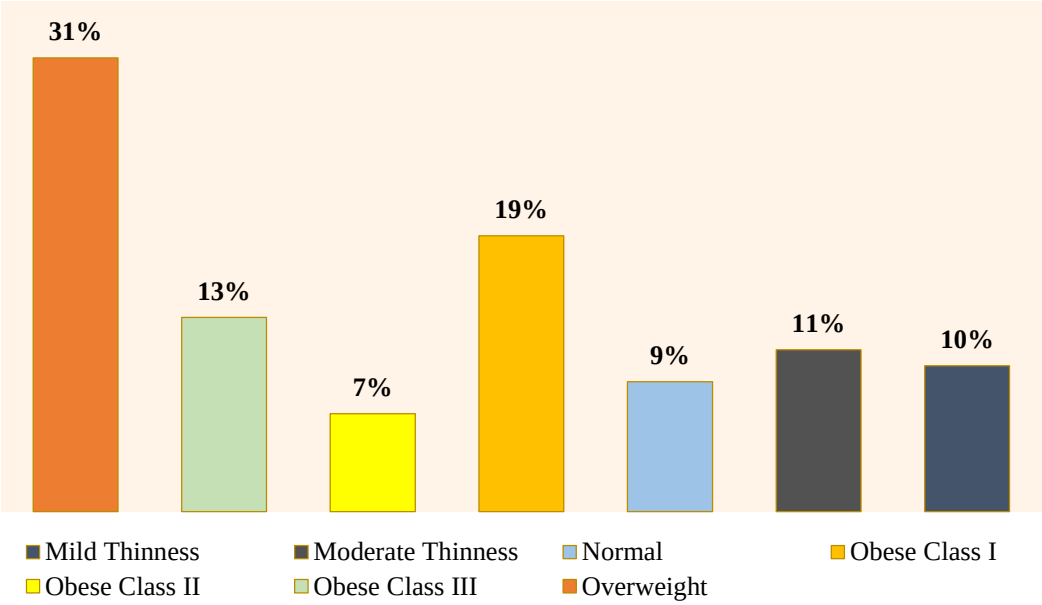


Figure (1): The categories of BMI of participants

Clinico – pathological factors according to DM types

Most of the positives patients were with DM-II as (31.1%) followed by DM-I as (21.1%) with significant differences regarding to the negative results of Adv-36 Abs ($p < 0.05$). In addition to, most of males were with DM-II as (33.3%) followed by females with DM-I as (27.8%) with significant differences between them ($p< 0.05$). There was a significant difference ($p < 0.05$) for DM-I patients of (8 – 25) of the age interval and regarding to the DM-II patients of (26 – 40) age interval. Seven categories of BMI was evaluated, as nine of (10%) patients were as mild thinness and all of them were with DM-I , 10 (11.1%) of patients were with moderate thinness with 8 (8.9%) patients had DM-I and 2 (2.2%) patients had DM-II, 28 (31.1%) over weight patients with 20 (22.2%) individuals had DM-II and 8 (8.9%) patients with DM-I with significant differences among all the rest of BMI categories classification ($p < 0.05$) which illustrate in table (2).

Table (2): Comparison between DM –I and DM-II according to Clinico- pathological factors

Characteristics		DM-I		DM-II		*p.value*
		NO.	%	NO.	%	
Adv – 36 antibodies	+ ve	19	21.1%	28	31.1%	0.05 ≤ 0.05 S*
	- ve	26	28.9%	17	18.9%	
	Total	45	50%	45	50%	
Genders	Male	20	22.2%	30	33.3%	0.03 < 0.05 S*
	Female	25	27.8%	15	16.7%	
	Total	45	50%	45	50%	
Age groups	(8 - 25)	25	27.8%	12	13.3%	0.004 < 0.05 S*
	(26 - 40)	7	7.8%	20	22.2%	
	≥ 41	13	14.4%	13	14.5%	
	Total	45	50%	45	50%	
BMI categories	Moderate Thinness	8	8.9%	2	2.2%	0.002 < 0.05 S*
	Mild Thinness	9	10.0%	0	0%	
	Normal	2	2.2%	6	6.7%	
	Overweight	8	8.9%	20	22.2%	
	Obese Class I	7	7.8%	10	11.1%	
	Obese Class II	4	4.4%	2	2.2%	
	Obese Class III	7	7.8%	5	5.6%	
	Total	45	50%	45	50%	

*p. value ≤ 0.05 (Significant)

Clinico – pathological factors according to Adv-36 Abs ELISA results

It was with higher percentages about (31.1 %) of DM-II cases whose had been with positive Adv-36 Ab , while it shows less with about (21.1%) of DM-I cases had been infected with adenovirus-36 with significant difference between them (p< 0.05). Moreover, the positive percentages of Adv-36 Abs distributed in females as representing with (30%), While it showed less percentages in males representing with (22.2%) all were in significant differences (p< 0.05) founded between study groups and genders. Furthermore, high positive percentages of Adv-36 Abs representing by (21.1%) of cases whose ages interval were limited between (8 – 25) years. While it shows less percentage in the age interval of (≥ 41) years by (13.3%) with significant difference (p< 0.05) were founded between study groups and age categories. Finally, the Adv-36 Ab presence with the seven categories of BMI showed, 10 (11.1%) over weight patients had Adv-36 Ab, 7 (7.8%) patients were Obese Class III had been detected with Adv-36 Ab, five (5.6%) subjects with Obese Class II had Adv-36 Ab, and 11 (12.2%) subjects Obese Class I had Adv-36 Ab, 7 (7.8%) patients were with normal BMI had been infected with Adv-36 Ab, 5 (5.5%) of patients were moderate thinness had been noticed with Adv-36 Ab, and 2 (2.2%) patients were as mild thinness were with Adv-36 Ab in significant differences with the absence of Adv-36 Abs in all BMI categories (p< 0.05) as presented in table (3).

Table (3): Comparison between Adv-36Ab ELISA results according to the studied Clinico- pathological factors

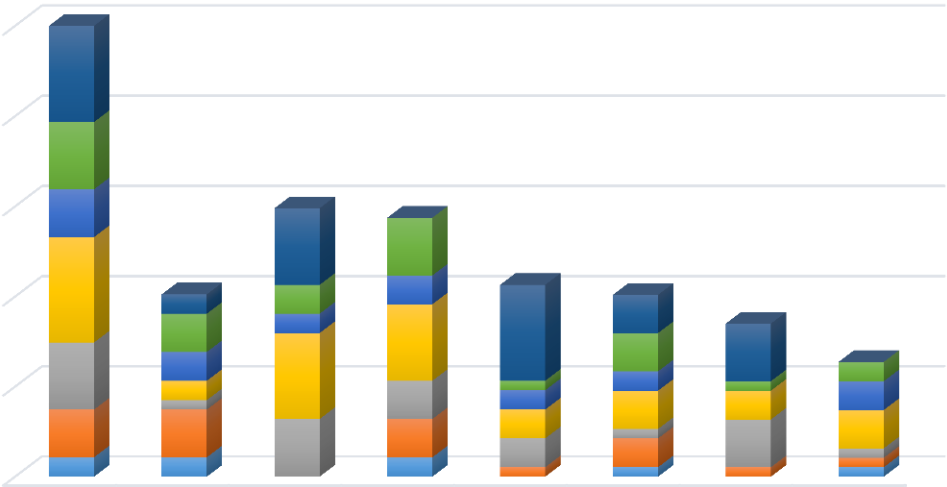
Characteristics		(+ ve) Adv-36Ab		(- ve) Adv-36Ab		p.value
		NO.	%	NO.	%	
DM - Types	DM-I	19	21.1	26	28.9	0.05 ≤ 0.05 S*
	DM-II	28	31.1	17	18.9	
	Total	47	52.2	43	47.8	
Genders	Male	20	22.2	30	33.3	0.009 < 0.05 S*
	Female	27	30.0	13	14.5	
	Total	47	52.	43	47.8	
Age groups	(8 - 25)	19	21.1	18	20.0	0.6 > 0.05 NS**
	(26 - 40)	16	17.8	11	12.2	
	≥ 41	12	13.3	14	15.6	
	Total	47	52.2	43	47.8	
BMI categories	Moderate Thinness	5	5.5	5	5.6	0.03 < 0.05 S*
	Mild Thinness	2	2.2	7	7.8	
	Normal	7	7.8	1	1.1	
	Overweight	10	11.1	18	20.0	
	Obese Class I	11	12.2	6	6.7	
	Obese Class II	5	5.6	1	1.1	
	Obese Class III	7	7.8	5	5.5	
	Total	47	52.2	43	47.8	

*p. value ≤ 0.05 (Significant), *p. value > 0.05 (non-significant)

Interpretation of BMI categories regarding to the studied Clinico – pathological factors

Figure (2): Explained the frequency distribution of the only (+ve Adv-36 Abs) ELISA results that represented in (47 patients) and it considered as (47 \ 100%) by discarding the negatives results for more easily statistical descriptions.

The first highest percentages of positives Adv-36 Abs were detected in Obese Class I patients as (23.4%) and most of them had been detected with DM-II by (19.1%), they were distributed with high percentages in females compared to the males by (17%) in high tendency as (8.5%) to the each of (8-25) and (26-40) age intervals. The second highest percentages of positives Adv-36 Abs were founded in overweight patients (21.3%) and most of them had been detected with DM-II as (17%) all of them were males (21.3%) in high tendency to the (26-40) of age interval. In the other hand , the lowest percentages of positives Adv-36 Abs were noticed in mild thinness patients (4.3%) had been detected with DM-I as (4.3%), all of them were females (4.3%) and distributed into the (8-25) and (≥ 41) of age intervals as (2.10%) for each group.



	Adv-36	DM - I	DM - II	Female	Male	(8-2)	(26-40)	≥ 41
Overweight	21.3%	4.3%	17.0%	0.0%	21.3%	8.5%	12.8%	0.0%
Obese Class III	14.9%	8.5%	6.4%	12.8%	2.1%	8.5%	2.1%	4.3%
Obese Class II	10.6%	6.4%	4.3%	6.4%	4.3%	4.3%	0.0%	6.4%
Obese Class I	23.4%	4.3%	19.1%	17.0%	6.4%	8.5%	6.4%	8.5%
Normal	14.9%	2.1%	12.8%	8.5%	6.4%	2.1%	10.6%	2.1%
Moderate Thinness	10.6%	10.6%	0.0%	8.5%	2.1%	6.4%	2.1%	2.1%
Mild Thinness	4.3%	4.3%	0.0%	4.3%	0.0%	2.10%	0.00%	2.10%

Figure (2): Frequency distribution of BMI according the studied Clinico – pathological factors

Discussion

In 1978 human adenovirus 36 (Adv36) was first identified and isolated [11], it belongs to 54 known human adenovirus strains group generally associated to some diseases in the respiratory tract or gastrointestinal tract or even more in the eyes (conjunctiva). All the 54 serotypes are classified into seven species based on their nucleic acid properties, immunochemical interactions, fiber and hexon protein individuality, the biological property, and the phylogenetic investigations, regarding to the Adv-36 that belonging to the adenovirus D subgroup (Adv-D), Obesity through increasing propagation and differentiation of preadipocytes and lipid accumulation in mature adipocytes it has been established that in vitro and in vivo Adv-36 infection of numerous animal types [2,3]. Most consequent reports on Adv-36 about obesity in human beings prove the humans with substantiation of prior, natural Adv-36 infection are heavier than uninfected individuals for obesity initiation. The statistics and data about the relationship of Adv36 with obesity in adults vary and many studies that being somewhat conflicting, but there was conclusion in children consistently correlate Adv-36 infection with obesity [4-12].

In dissimilarity to the majority cases of obesity, Adv36 linked obesity has related to lower levels of blood lipid profile in animals and adult humans. Also, Adv36 is noticed to develop glycemic control in chow-fed and in high-fat diet-fed mice by attractive glucose uptake in skeletal muscle, adipose tissue and reduce hepatic glucose release. Additional, The Adv36 was linked with enhanced insulin sensitivity in most of obese individuals and overweight [12, 13, 14].

That (Adv-36) obesity relationship in earlier findings is less reliable in adults than in children ages intervals maybe due to, other overweight/obesity risk factors, as life style, that may have had a longer time to be shows in adults than in younger ages, and therefore mask an (Adv-36) outcomes in adult's life. As well, patients with younger ages may have had minimum chance to build up an immune reaction

against Adv-36 infection by contact to other Adv36-cross reacting adenoviruses serotypes. Further, the antibody response to vaccination processes against viral infection has been shown to decreased with age and BMI, although there is no evidence for elevated antibody titer against Adv-36 in young ages regarding to in old individuals. However, it is possible that an Adv-36 antibody titter in a person decreases to invisible level so. For example, a decline in the antibody titer and T-cell response during the antiviral vaccination of first year after was more distinct in obese adult ages [7-12-15].

Preceding conclusions from in vitro and in vivo studies of human tissue and in chicken respectively, experimental infection of rodents and primates show that with Adv36 induces preadipocytes proliferation, fat accumulation in cells, , adipocytes segregation, and body fat raised by viral E4orf1 protein (early region 4 open reading frame 1, Adv) mediates the Adv36 consequences including its adaptogenic potential [7-15].

Despite this, many reports explored the association of virus infection with heart problems, blood pressure issues, allergic disorders, autoimmune diseases or even cancers that had been carried out [16-20]. The (Adv36) progressed adiposity and upregulates signaling of distal insulin in vitro and in vivo, need further studies to study the impacts with diabetes problems.

Recommendation:

Conduct a large cohort study recruiting a large number of obese , DM-I & DM-II Iraqi patients to reinforce the results of the current study which may lead to a better understanding the biology of this issue with adding advanced molecular techniques for virus detection.

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