Study the effect of obesity on the level of adiponectin and DNA damage

in obese patients with type2 diabetes in Wasit Province

Received :31/1/2018

Accepted : 27 / 5 /2018

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Abstract

The study present was conducted on 36 patients of both sexes with diabetes type 2 (DM2) obese aged (30-62) years. The body mass index (BMI) of those patients was \geq 30 kg/m and compared to 2 groups: the first included 20 people who are non-diabetic non-obese of age (30-75) years and the second included 26 people who are non-diabetic obese with ages (22-67) years. The level of adiponectin in patients and both control groups was measured. The results showed a significant decrease in adiponectin when compared to DM2 obese and non-diabetic non-obese, while non-significant decrease were found when compared to non-diabetic type 2 obese. The results of the percentage of DNA damage of lymphocytes in patients when using comet assay test have been found to be significance (P \leq 0.05) in DM2 obese using Tail Mean Moment and Tail Length (Px) parameters when compared to both control groups. Also the results showed high significance (P< 0.05) in

other parameters such as Olive Moment, DNA in Head (%) and DNA in Tail (%) in type2 diabetic obese group as compared to non-diabetic non-obese group.

Key words: Diabetes mellitus, Obesity, Adiponectin, Comet assay

Introduction

DM2 mellitus defined as a disturbance in the glucose metabolism that leads to increase its level in the blood (1) and characterized usually by insulin resistance (IR). It is considered one of the genetic diseases especially for those who have diabetic patients of close relationship (2). IR is closely related to the obesity and sedentary situations as have been established in many studies (3). Several studies mentioned that diabetes mellitus resulted from high income that might encourage low activity, laziness, anxiety, and pressure which might be even worse with obesity (4). The relation between fat cells hyperplasia and obesity is considered the

Main reason of IR (5), and due to the fact that adiponectin which is a protein hormone that is exclusively secreted by adipocytes adipose tissue. or Adiponectin is an anti-inflammatory hormone that might decrease the risk of DM2 (6). In addition, there is an obesity impact on the heart health and metabolism (7). Studies refer to the role of adiponectin in regulation of insulin path in the body and it also works to decrease the glucose production by the liver (8). Lately, there is an advance in understanding the genetic factors due to the usage of new techniques that help to figure the DNA sequence and the genetic errors. Alkaline Comet Assay has been used to detect the genetic errors at the DNA level in the lymphocytes (9). Comet Assay is a single cell gel electrophoresis that considered simple and feasible assay to detect genetic errors in eukaryotic cells (10).

The current study aimed to study the effect of both DM2 and the obesity on adiponectin level and to evaluate genetic errors that might be resulted from both conditions.

Materials and Methods

This study had been done in College of Sciences, University of Wasit. Venous blood samples were collected from DM2 patients in the Diabetes and Endocrine centers/Al-Zahraa Educational Hospital and Al-Karamah Hospital/ Wasit city.

Adiponectin Evaluation

Adiponectin levels were evaluated using the Mini vietric Immuno-diagnostic assay device (Minividas) (Biomerieux, France).

Alkaline Comet Assay

Alkaline Comet assay is the most sensitive method of the Comet assays, and so it can detect the minor breaks such as double strand breaks and single strand breaks. Comet assay which is also known as DNA gel electrophoresis was performed in this study according to Klaude et al. (11). The Comet assay used here to detect the DNA breaks in lymphocytes of patients that suffered type2 diabetes and obesity in addition to control groups. This technique includes packaging the cells with low Melting Agarose (LMAgarose of low melting point). Then, the cells were lysed under (pH>13) condition to start the assay. The assay depends on the ability of the damaged DNA to migrate to out of the nucleus due to the effect of the electric field. While the intact DNA either migrates slowly or stays inside the nuclear field. The Comet assay evaluates the level of the DNA damage by evaluating the movement ability and length and shape of the comet itself (12). Then, the DNA that was stained previously with SYBR Green stain analyzed using the Comet Score software as shown in Figure 1.



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Comet Length (px) Comet Height (px) 602.00000 149.00000 72613.00000 556.00000 207.00000 74632.000000 765.000000 175.00000 86072.00000 401.00000 155.00000 51224.00000 299.00000 180.00000 46660.00000 345.00000 189.00000 43431.00000	Comet Area (px) Comet Intensity Comet Mean Intensity Head Diameter (7204100.00000 99.212265 486.00000 67220.00000 6839577 6882736.000000 92.222321 494.000000 74432.000000 6870374 8221136.000000 95.514641 708.000000 85753.000000 8203858 5097559.000000 95.515053 401.000000 51223.000000 5097558 4811803.000000 103.124794 298.000000 46558.000000 4811160 4087004.000000 94.103386 345.000000 43397.000000 4087003	PX) * .000i .000i .000i .000i .000i
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Figure 1: Comet analysis using the Comet Score software.

Statistical Analysis

To eva	luate	the	statistical	differences
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between the groups, Statistical Package

for Social (SPSS 13) was used (13).

Results

Adiponectin levels study:

The adiponectin levels study showed that adiponectin levels were significantly lower in DM2 obese patients compared to DM2 non-obese non-diabetic non-

obese ones (P ≤ 0.05) as shown in Table	been registered significant difference (P
1. While there were no difference	<0.05) in adiponectin levels between the
between obese patients with DM2 and	two control groups themself.
obese non DM2. Also the results have	

Table 1: Adiponectin levels in diabetic type2 obese, non-diabetic non-obese, and non-diabetic obese patients in Wasit province.

Treatments	Diabetic type2	Non-Diabetic non-	Non-Diabetic
	obese	obese	Obese
	N = 36	N = 20	N = 26
	$BMI \ge 30 \text{ kg/m2}$	BMI=(25-29.9)	$BMI \ge 30 \text{ kg/m2}$
Parameter		kg/m2	
	a	b	а
Adiponectin	$\textbf{3.47} \pm \textbf{3.27}$	$\textbf{7.97} \pm \textbf{3.70}$	4.19 ± 3.74
hormone (µg/ml)			



DNA damage study:

In table 2, the results showed that significant increase in the parameters that were used to evaluate the DNA damage levels in obese patients who suffered DM2 and obesity Tail Mean Moment and (Tail Length (Px) compared to the control groups. The results also showed that there were differences in the other parameters (Olive Moment and (%) DNA in Head and (%) DNA in Tail at (P \leq 0.05) compared to non-diabetic non-obese patients and non-significant differences compared to the non-diabetic obese patients. On the other hand there is nonsignificant differences p> 0.05 between obese patients with DM2 and non-DM2

obese

Table 2: The DNA damage levels in obese patients with DM2, non-diabetic non-obese,

 and non-diabetic obese by using Comet assay in Wasit city.

\mathbf{N}	Alkaline Comet Assay		
Treatments	Diabetic type2 obese	Non-Diabetic non-obese	Non-Diabetic Obese
	N = 36	N = 20	N = 26
	BMI ≥ 30 kg/m2	BMI=(25-29.9) kg/m2	BMI ≥ 30 kg/m2
Parameter			
Tail Mean	а	b	с
Moment	86.84 <u>+</u> 5.58	4.51 <u>+</u> 1.64	71.91 <u>+</u> 9.07
(mean <u>+</u> SD)			
Olive Moment	а	b	а
(mean <u>+</u> SD)	11.49+1.35	4.37+0.86	9.99+1.30
Tail Length(Px)	a	b	с
(mean <u>+</u> SD)	246.52 <u>+</u> 24.37	12.42 <u>+</u> 0.08	58.35 ± 7.64
DNA in	a	b	ab
Head(%)	66.05 <u>+</u> 3.54	85.46 <u>+</u> 2.04	73.85 <u>+</u> 1.72
(mean <u>+</u> SD)			
DNA in Tail (%)	а	b	а
(mean <u>+</u> SD)	20.66 <u>+</u> 1.62	6.23 <u>+</u> 0.75	18.74 <u>+</u> 1.79

Different letters refer to significant differences between the group at (P ≤ 0.05)



Figure 2: Comet assay image (100X) in lymphocytes of type2 diabetic obese patients after being stained with SYBR Green stain under Fluorescent microscope.

Discussion

Adiponectin levels study

The decrement in the levels of adiponectin in this study indicates that the Adiponectin is involved in glucose and lipid metabolism. It also works to increase fat oxidation and increase the sensitivity of insulin hormone as important factors to maintain healthy metabolism which indicates a relationship between obesity and insulin hormone (14). So, the level of adiponectin decreased in diabetic obese patients with insulin resistance. Many studies showed that obesity is synchronized with insulin resistance and low adiponectin levels (16).

DNA damage study

The results in table 2 Of Comet assay showed that there are significant differences (P<0.05) in the levels of DNA breaks in obese patients with DM2 as was previously shown by Blasiak *et al.*(16). The results showed the relation between the diabetes, obesity, and the level of DNA damage. This might be related to the IR, high level of glucose, and high level of lipid in the blood as it is known that there is a connection between the high level of glucose and the DNA damage (17). This case may lead to increase the level of the chromosomes injury in obese diabetic patients (18). This injury might be resulted from the high level of the glucose in the patients that in turn resulted from IR. The resulted free radicals might affect the DNA causing its damage because cells deal with the free radicals as toxic and mutated substances. So, the Alkaline Comet Assay is important in this regard since it measures the length of the DNA tail (comet) which indicates the damage level as was shown in figure2. Since the

length of the comet is proportionally related to the level of the DNA damage which ends up with oxidation, oxidative stress, and finally DNA damage. Fortunately, most of these damages can be fixed by the cells through the photorepair system, the system of reform by eradication, and cut and reform the new unions that make the genetic background one of the important diabetes risk factors although it hasn't been explored which gene is directly responsible for this yet.

Conclusions: It can be concluded that the level of adiponectin is affected by type2 diabetes and obesity and its level decreased according to the level of the disease and obesity. In addition, the level of DNA damage is increased as a result of type2 diabetes and obesity.

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