Impact of weight loss on selected fibrinolytic parameters, endothelial and platelets microparticles in non-insulin dependent diabetic patients

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Abstract: Background: Currently the prevalence of diabetes is gradually increasing, and vascular complications associated with diabetes seriously affect patients' health as the elevated level of endothelial and platelets microparticles (EMP and PMP) was associated with vascular dysfunction in diabetic patients. The effectiveness of intentional weight loss in reducing cardiovascular disease (CVD) events in type 2 diabetes is unknown. However, the influence of adiposity and weight reduction on PMP and EMP generation remain to be fully elucidated. **Objective:** This study was an attempt to measure the effects of weight loss on fibrinolytic parameters (plasminogen activator inhibitor-1 activity (PAI-1: Ac), tissue plasminogen activator antigen (tPA:Ag) and fibrinogen & endothelial and platelets microparticles (CD41 and CD144) in non-insulin dependent diabetic patients. **Methods:** Eighty non-insulin dependent diabetic patients (42 males, and 38 females) with mean age 37.86± 5.21 years were divided into two equal groups; the training group received diet regimen, exercise training in addition to their medical treatment for two months, where the control group received their medical treatment only. **Results:** There was a 13.1%, 26.4%, 14.5%, 26.8%, 12.7% and 13.5% reduction in mean values of BMI, tPA: Ag, fibrinogen, PAI-1: Ac, PMP and EMP respectively in group (A). While, there was a 1.7%, 5.2%, 3.7%, 5.7%, 2.5% and 2.9% reduction in mean values of BMI, tPA: Ag, fibrinogen, PAI-1: Ac, PMP and EMP in group (B). The mean values of BMI, tPA: Ag, fibrinogen, PAI-1: Ac, PMP and EMP were decreased significantly in the group (A), however the results of group (B) were not significant. Also, there were significant differences between both groups at the end of the study. **Conclusion:** Weight loss modulates fibrinolytic parameters, endothelial and platelets microparticles in non-insulin dependent diabetic.
[Shehab M. Abd El- Kader, Mohamed S. Al-Dahr, Amer A. Alsaif, Samira Alsenany. **Impact of weight loss on selected fibrinolytic parameters, endothelial and platelets microparticles in non-insulin dependent diabetic patients.** *Life Sci J* 2014;11(2s):79-85]. (ISSN:1097-8135). http://www.lifesciencesite.com. 14

Key words: Non-Insulin Dependent Diabetes; Weight Loss, Fibrinolytic Parameters, Endothelial Microparticles; Platelets Microparticles.

Introduction

Diabetes mellitus affects 285 million people worldwide, with numbers predicted to rise by 54% over the next 20 years [1]. However, a recent analysis revealed that men with diabetes but without a history of cardiovascular disease at 40, 50, and 60 years of age will die approximately 6.3, 5.8, and 4.5 years, respectively, earlier than men without diabetes [2]. Moreover, the costs associated with pre-diabetes and diabetes reached $218 billion in 2007 in the U.S. alone, which included $153 billion in medical costs and $65 billion in reduced productivity [3].

Microparticles (MP) are fragments of virtually all cell types (endothelium, platelets, leukocytes) released during cell apoptosis or activation and characterized by an integral plasma membrane expressing the phenotype of the cell from which they originated [4]**.** Endothelial cells (EMP) or platelets (PMP) that may actively modulate inflammation, coagulation and vascular function [5&6].

Although a few microparticles can be detected in healthy people, the circulating level of microparticles significantly increases in diabetes, which suggests that microparticles may be involved in multiple pathophysiological processes in the body, such as thrombosis, inflammatory condition and atherogenesis [7]. Elevation of plasma MPs levels, particularly those of endothelial origin, reflects cellular injury and appears now as a surrogate marker of vascular dysfunction [8]**.**

The circulating level of microparticles increases in patients with type 2 diabetes. The level of endothelial microparticles is closely associated with vascular dysfunction [9]**.** High level of endothelial microparticles in patients with diabetes may accelerate the decline of arterial elasticity [10&11]. Microparticles (MP) are associated with most CVD risk factors and appear indicative of a poor clinical outcome [12&13]**.** Finally, MP represent pathogenic vectors able to propagate noxious responses including

pro-coagulant and pro-inflammatory pathways, vascular remodelling and endothelial dysfunction [14].

The incidence of cardiovascular disease is at least three times higher in diabetic than in non-diabetic populations, and 50–75% of diabetic patients die from coronary artery disease (CAD) [15&16]. Coronary artery lesions are more extensive and CAD has a worse prognosis than in non-diabetic subjects [17]**.** Moreover, several studies have demonstrated that increased plasma levels of plasminogen activator inhibitor-1 (PAI-1) and tissue-type plasminogen activator (t-PA) are independent predictors of CVD as atherothrombosis [18-21]**.** Therefore weight loss in obese patients can be considered a very effective strategy to improve the platelet abnormalities linked to insulin resistance [22]**.**

Lifestyle modification in the form of caloric restriction and increased physical activity are the most common modalities used for treating non-insulin dependent diabetic patient. However, the influence of adiposity and weight reduction on PMP generation remains to be fully elucidated. So, this study was an attempt to measure the effects of weight loss on fibrinolytic parameters, endothelial and platelets microparticles response to weight reduction in non-insulin dependent diabetic patients.

Subjects, Methods

Subjects

Eighty obese non-insulin dependent diabetic patients as defined in accordance with the criteria of the American Diabetes Association [23&24]**,** their body mass index (BMI) ranged 30-35 kg/m^2 was recruited for this study at the Diabetes Clinic of King Abdul-Aziz University Teaching Hospital, their age was ranged from 40 to 55 years. All volunteers were asked to read and sign an informed consent document prior to participation. To minimize confounding factors, we selected a homogeneous patient population with respect to the degree of metabolic control (hemoglobin A1C (HbA1c) < 8%).

Subjects were excluded if they had a history of smoking during the previous 6 months or if they had a history of cardiovascular disease (angina pectoris, recent myocardial infarction, history of coronary artery bypass surgery or angioplasty, congestive heart failure, or arrhythmia), stroke, or transient ischemic attacks. Other exclusion criteria included total cholesterol (TC) >300 mg/dl, triglycerides >600 mg/dl (untreated or treated), blood pressure >160/100 mmHg (untreated or treated), FPG >300 mg/dl, or HbA1c >11%. Subjects with diabetic proliferative retinopathy, nephropathy (albumin excretion >300 µg/mg creatinine and/or serum creatinine >2.0 mg/dl), signs or symptoms of diabetic autonomic or peripheral neuropathy.

Participants were divided into two equal groups; the first group (A) received physical training combined with dietary measures and their medical treatment, where the second group (B) received their medical treatment and asked to maintain their ordinary life style without physical therapy intervention.The program consisted of three sessions per week for three months. This study was approved by the Scientific Research Ethical Committee, Faculty of Applied Medical Sciences at King Abdulaziz University.

Methods

A. Laboratory analysis:

- Blood collection

Five milliliter of blood was collected from the antecubital vein between 8 and 10 am after an overnight fast and at rest using the two-syringe method. The first sample was drawn into polypropylene tube for serum collection. The second sample was gently introduced into a polypropylene tube containing 1/10 volume of 3.13% sodium citrate (Vacutainer, Becton Dickinson); for platelet-poor plasma (PPP), obtained by centrifugation at 2000 ×g for 10 min at 4 °C and was stored at − 80 °C until analysis.

- Analysis of fibrinolytic parameters

The levels of PAI-1 activity and tissue-type plasminogen activator (t-PA) antigen were determined from the PPP samples using a commercial kit (Hyphen BioMed for PAI-1 and t-PA, France). Fibrinogen was measured by the time titration method employing the ST-4 coagulation instrument (Zymutest Fibrinogen, ELISA, Hyphen Biomed, Neuville sur Oise, France).

- Microparticle preparation and quantification

Microparticle preparation and quantification were processed according to the International Society of Thrombosis and Hemostasis (ISTH) recommendations for microparticles analysis [25], 30 µl of platelet poor plasma was incubated for 20 min in the dark with 4 µl of fluorescein (FITC)–conjugated monoclonal antibody against CD41 (GPIIb) (Beckmann) or 6 µl of phycoerythrin (PE)-conjugated monoclonal antibody against CD144 (VE-Cadherin) (Beckmann) for determination of platelet and endothelial MP, respectively. After incubation, samples were diluted in 900 ml of PBS. To assess non-specific binding of antibodies, non-immunized FITC- and PE-labeled isotype-matched mouse monoclonal IgG were served as negative controls. Immediately before flow cytometry analysis 30 µl of flow count beads (CXP FC500 Beckmann Coulter) was added to microparticle quantitation [26]**.**

Measurements of fibrinolytic parameters (plasminogen activator inhibitor-1 activity (PAI-1: Ac), tissue plasminogen activator antigen (tPA:Ag) and fibrinogen & endothelial and platelets microparticles (CD41 and CD144) were taken before the starting of the study (pre-test) and after three months at the end of the study (post-test).

2. Controlling nutrition

All subjects of group (A) were instructed to take an individual balanced energy-restricted dietary program to obtain weight loss.The mean daily caloric intake was about1200 kcal/day, based on a macronutrient content <30% fat and 15% protein as recommended by the World Health Organization [27]**.** At the initial interview with a dietitian, obese subjects was given verbal and written instructions on how to keep diet records, with food weighed and measured. Dietary intake was monitored by the same dietitian. The subjects maintained a detailed record of food intake, and also received weekly nutritional counseling. Obese subjects were instructed to substitute low-fat alternatives for typical high-fat foods, to increase the consumption of vegetables and fresh fruits, and to substitute complex carbohydrates, such as whole-grain bread and cereals. Dietetic help was given every 2 weeks by the dietitian when anthropometric measurements were performed; in addition, each subject was seen by a physician monthly to perform a clinical evaluation, standard electrocardiogram, and measurement of blood pressure and heart rate [28&29].

3. Exercise training program

All subjects of group (A), in addition diet control, performed aerobic exercise training 3 days per week (40 min per session), supervised by physical therapist (the initial, 5-minute warm-up phase performed on the treadmill (Track master 400E, gas fitness system, England) at a low load, each training session lasted 30 minutes and ended with 5-minute recovery and relaxation phase) either walking or running, based on heart rate, until the target heart rate was reached, according to American College of Sport Medicine guidelines. The program began with 10 min of stretching and was conducted using the maximal heart rate index (HRmax) estimated by: 220-age. First 2 weeks = 60–70% of HRmax, 3rd to 12th weeks = 70–80% of HRmax. The performance of the subjects was controlled by a physical education expert and their heartbeat was constantly checked by the polar device (POX 1000 Japan), and the control group remained sedentary in this period [28].

Statistical analysis

The mean values of BMI, tPA:Ag, fibrinogen, PAI-1:Ac, PMP CD41 + and EMP CD144 + were compared using paired "t" test. Independent "t" test was used for the comparison between the two groups ($P<0.05$).

Tables

Table 1. Comparisons of baseline anthropometric and serum or plasma biochemical data in group (A) vs. group (B).

	Mean ± SD		p value
	Group A (N = 40)	Group B (N = 40)	
Age,years	48.32 ± 6.87	47.42 ± 6.95	0.271
Body weight,kg	95.61 ± 7.52	96.23 ± 5.31	0.563
BMI,kg/m^2	32.81 ± 2.53	32.41 ± 2.23	0.483
Waist circumference,cm	105.11 ± 8.46	104.32 ± 8.50	0.176
Duration of diabetes,years	10.83 ± 2.35	10.63 ± 2.27	0.135
Systolic blood pressure, mm Hg	133.21 ± 10.26	132.29 ± 10.56	0.315
Diastolic blood pressure, mm Hg	85.93 ± 7.13	83.87 ± 7.85	0.272
Fasting glucose,mg/dl	108.16± 9.15	107.24 ± 9.08	0.361
Fasting insulin,µIU/ml	18.46 ± 5.83	18.72 ± 6.22	0.841
Total cholesterol,mg/dl	218.17 ± 8.34	216.89 ± 7.23	0.582
HDL-cholesterol, mg/dl	42.65 ± 5.28	41.97 ± 5.76	0.626
LDL-cholesterol, mg/dl	137.41 ± 7.52	136.31 ± 8.11	0.231
Triglyceride,mg/dl	287.36 ± 15.8	286.32 ± 13.89	0.027
HbA1c,%	7.65 ± 1.84	7.82 ± 1.91	0.214
PAI-1 activity, ng/ml	0.56 (0.35–1.08)	0.53 (0.32–1.02)	0.081
t-PA antigen, ng/ml	6.98 (5.78–7.92)	6.78 (5.46–7.54)	0.063
Fibrinogen, mg/dl	308.15 ± 8.73	306.62 ± 8.19	0.831
PMP CD41 +,counts × 10^3/ml	186.17 [157.23–216.34]	185.43 [154.11–211.82]	0.033
EMP CD144 +,counts × 10^3/ml	258.60 [127.11–482.42]	255.17 [128.51–476.50]	0.054

BMI = Body Mass Index; HDL= High Density Lipoprotein
LDL= Low Density Lipoprotein; HbA1c = Hemoglobin A1C
PAI-1: Ac = Plasminogen Activator Inhibitor-1 Activity
tPA: Ag = tissue Plasminogen Activator Antigen
PMP = Platelet Microparticle; EMP= Endothelial Microparticle

Table 2. Mean value and significance of the pre and post test values of BMI, tPA:Ag, fibrinogen, PAI-1:Ac, CD41 + and EMP CD144 + of group (A).

	Mean ± SD		t- value	p value
	Pre	Post		
BMI,kg/m^2	32.81 ± 2.53	28.52 ± 1.87	6.51	0.021
t-PA antigen, ng/ml	6.98 ±1.98	5.14 ±1.86	6.13	0.015
Fibrinogen, mg/dl	308.15 ± 8.73	263.36 ± 8.14	8.42	0.003
PAI-1 activity, ng/ml	0.56 ±0.14	0.41 ±0.12	4.27	0.035
PMP CD41 +,counts × 10^3/ml	186.17 ±15.45	162.47 ±13.61	8.63	0.002
EMP CD144 +,counts × 10^3/ml	258.60 ±18.92	223.54±16.15	9.42	0.001

BMI = Body Mass Index
tPA: Ag = tissue Plasminogen Activator Antigen
PAI-1: Ac = Plasminogen Activator Inhibitor-1 Activity
PMP = Platelet Microparticle EMP= Endothelial Microparticle

Table 3. Mean value and significance of the pre and post test values of BMI, tPA:Ag, fibrinogen, PAI-1:Ac, CD41 + and EMP CD144 + of group (B).

	Mean ± SD		t- value	p value
	Pre	Post		
BMI,kg/m^2	32.41 ± 2.23	31.85 ± 2.16	0.56	0.92
t-PA antigen, ng/ml	6.78 ±1.65	6.43 ±1.51	0.97	0.85
Fibrinogen, mg/dl	306.62 ± 8.19	295.23 ± 8.15	1.14	0.07
PAI-1 activity, ng/ml	0.53 ±0.16	0.50 ±0.17	0.46	0.64
PMP CD41 +,counts × 10^3/ml	185.43 ±13.64	180.81 ±12.95	1.25	0.08
EMP CD144 +,counts × 10^3/ml	255.17±17.51	247.84±16.86	1.73	0.06

BMI = Body Mass Index
PAI-1: Ac = Plasminogen Activator Inhibitor-1 Activity
tPA: Ag = tissue Plasminogen Activator Antigen
PMP = Platelet Microparticle; EMP= Endothelial Microparticle

Table 4. Mean value and significance of BMI, tPA:Ag, fibrinogen, PAI-1:Ac, CD41 + and EMP CD144 + in group (A) and group (B) after treatment.

	Mean ± SD		t- value	p value
	Group (A)	Group (B)		
BMI,kg/m^2	28.52 ± 1.87	31.85 ± 2.16	5.31	0.023
t-PA antigen, ng/ml	5.14 ±1.86	6.43 ±1.51	5.07	0.024
Fibrinogen, mg/dl	263.36 ± 8.14	295.23 ± 8.15	7.24	0.006
PAI-1 activity, ng/ml	0.41 ±0.12	0.50 ±0.17	4.15	0.037
PMP CD41 +,counts × 10^3/ml	162.47 ±13.61	180.81 ±12.95	7.23	0.008
EMP CD144 +,counts × 10^3/ml	223.54±16.15	247.84±16.86	8.17	0.005

BMI = Body Mass Index
PAI-1: Ac = Plasminogen Activator Inhibitor-1 Activity
tPA: Ag = tissue Plasminogen Activator Antigen
PMP = Platelet Microparticle; EMP= Endothelial Microparticle

Results

The two groups were considered homogeneous regarding the Baseline descriptive characteristics (Table 1). There was a 13.1%, 26.4%, 14.5%, 26.8%, 12.7% and 13.5% reduction in mean values of body mass index (BMI), tissue Plasminogen Activator Antigen (tPA: Ag), fibrinogen, Plasminogen Activator Inhibitor-1 Activity (PAI-1: Ac), Platelet Microparticle (PMP) and Endothelial Microparticle (EMP) respectively in group (A) (Table 2). While, there was a 1.7%, 5.2%, 3.7%, 5.7%, 2.5% and 2.9% reduction in mean values of BMI, tPA: Ag, fibrinogen, PAI-1: Ac, PMP and EMP in group (B). The mean values of BMI, tPA: Ag, fibrinogen, PAI-1: Ac, PMP and EMP were decreased significantly in the group (A), however the results of group (B) were not significant (Table 3).

Also, there were significant differences between both groups at the end of the study (Table 4).

Discussion

Diabetes is a debilitating disease and is often associated with obesity, so it is imperative to develop novel therapeutics to combat the associated obesity as cardiovascular disease is the primary cause of death in diabetic patients [30] It is well-documented in the literature that microparticles are increased in diabetes [31] and involved in the pathogenesis of diabetic complications, so microparticles could be considered to be important markers of cardiovascular risk [32]. Expert guidelines for primary prevention of cardiovascular disease in diabetes mandate weight loss as an important part of risk reduction [33]. To our knowledge, however, only few trials investigated at present whether the changes of lifestyle may influence the prothrombotic tendency in obese subjects [29&34] and even fewer trials investigated the effects on platelet function [35].

The principal finding of this study is that a 3-month program of lifestyle modification in the form of caloric restriction and physical exercise in obese type 2 diabetic subjects significantly improved microparticles and fibrinolytic parameters. This improvement was strongly associated with weight reduction. This effect was also associated with significant reduction in the plasma levels of selective markers of endothelial, platelets microparticles and coagulation. These findings are supported by many previous studies on obese subjects that found that weight reduction regimens either dietary therapy, physical activity and combination therapy (diet and physical activity) contribute to a decrease in CVD-related morbidity through improvement of fibrinolytic abnormality and endothelial dysfunction [36-39].

In this study, the high values of plasma PAI-1 activity and t-PA antigen levels were reduced after weight reduction in diabetic obese subjects. This association was in line with data of previous weight reduction trials [37-39], could be explained by reduction of TNF level secreted by adipocytes [40]. Also, moderate exercise intensity may also suppress platelet activation and polymorphonuclear leukocyte interaction with surface-adherent platelets under shear flow [41].

The underling mechanism of reduction in microparticles production as a result of weight loss in patients with type 2 diabetes mellitus reflects an attenuation of the low-grade inflammation, which is induced by adipose tissue. These facts support the idea that excessive adipocytes that lead to low-grade inflammation possibly play an important role in PMP production. Another possible explanation for PMP generation in obesity is the association of other adipokines such as leptin, because they increase in obesity and diminish after weight loss in proportion with BMI [42]. Additionally, high concentrations of leptin promote ADP-induced platelet aggregation, through leptin receptors expressed on the platelet surface [43&44]. Thus, increased level of leptin in obesity may influence ADP-induced PMP generation. This explaination is supported by Diamant et al. found that the values of microparticles correlate with BMI and TNF in uncomplicated type 2 diabetes mellitus [45]. Also, Nomura et al. reported that PMP levels correlated with the levels of IL-6 and IL-8 in patients with systemic inflammatory response syndrome [46]. However, the levels of these proinflammatory adipokines increase with increasing adipose tissue, and diminish after weight loss [47&48].

Conclusion

Weight loss improves markers of fibrinolytic parameters, endothelial and platelets microparticles in Saudi non-insulin dependent diabetic, so it is recommended to apply a weight reduction program to modulate fibrinolytic parameters, endothelial and platelets microparticles in non-insulin dependent diabetic.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

Acknowledgment

This work was funded by the Deanship of Scientific Research (DSR), King Abdulaziz University, Jeddah, under grant No. (142-003-D1434). The authors, therefore, acknowledge with thanks DSR technical and financial support.

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