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Erectile dysfunction and diabetes: Association with the impairment of lipid metabolism and oxidative stress

Arben Belba [a,1], Alessio Cortelazzo [b,*,1], Giansanti Andrea [a], Jacopo Durante [a], Laura Nigi [c], Francesco Dotta [c], Anna Maria Timperio [d], Lello Zolla [d], Roberto Leoncini [b], Roberto Guerranti [b], Roberto Ponchietti [a]

[a] Urological and Andrological Unit, Department of Medicine, Surgery and Neuroscience, University Hospital, Siena, Italy
[b] Department of Medical Biotechnologies, University of Siena, Siena, Italy
[c] Diabetes Unit, Department of Medicine, Surgery and Neurosciences, University of Siena, Siena, Italy
[d] Department of Ecological and Biological Sciences, University of Tuscia, Viterbo, Italy

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ABSTRACT

Objectives: To test the hypothesis that exists an association of non-diabetic and diabetic patients suffering from erectile dysfunction (ED) with lipid metabolism and oxidative stress.

Design and methods: Clinical and laboratory characteristics in non-diabetic (n = 30, middle age range: 41–55.5 years; n = 25, old age range: 55.5–73), diabetic ED patients (n = 30, age range: 55.5–75 years) and diabetic patients (n = 25, age range: 56–73.25), were investigated. Proteomic analysis was performed to identify differentially expressed plasma proteins and to evaluate their oxidative post-translational modifications.

Results: A decreased level of high-density lipoproteins in all ED patients ($P < 0.001$, C.I. 0.046–0.10), was detected by routine laboratory tests. Proteomic analysis showed a significant decreased expression ($P < 0.05$) of 5 apolipoproteins (i.e. apolipoprotein H, apolipoprotein A4, apolipoprotein J, apolipoprotein E and apolipoprotein A1) and zinc-alpha-2-glycoprotein, 50% of which are more oxidized proteins. Exclusively for diabetic ED patients, oxidative posttranslational modifications for prealbumin, serum albumin, serum transferrin and haptoglobin markedly increased.

Conclusions: Showing evidence for decreased expression of apolipoproteins in ED and the remarkable enhancement of oxidative posttranslational modifications in diabetes-associated ED, considering type 2 diabetes mellitus and age as independent risk factors involved in the ED pathogenesis, lipid metabolism and oxidative stress appear to exert a complex interplay in the disease.

1. Introduction

Erectile dysfunction (ED) is defined as the inability to achieve and maintain a penile erection adequate for satisfactory sexual intercourse [1]. It is a chronic condition that exerts a negative impact on male self-esteem and nearly all life domains including interpersonal and family relationships. ED is a common complaint in men over 40 years of age, and its prevalence rates increase throughout the aging period [2]. The pathogenesis of ED is usually multifactorial and involves organic, physiologic, endocrine, and psychogenic factors [3]. Major health concerns such as metabolic syndrome, hyperlipidemia and diabetes have become well integrated into the investigation of ED [4,5]. Metabolic syndrome is closely related to ED, and hyperlipidemia is considered to be a major risk factor [6]. The impairment of lipid metabolism in patients suffering from ED is an example of a link between this condition, atherosclerosis and other endothelial dysfunction [7]. Moreover, ED is the third most frequent complication of diabetes that affects the quality of life and it is often indicative of underlying vasculopathy representing a predictor of more serious cardiovascular disorders [8]. In young men (<45 years), it could represent one of the first clinical symptoms of a hyperglycemia or diabetes [9]. In diabetes-associated ED, oxidative stress (OS)-mediated neurovascular alterations can play an integral role, inducing impaired endothelial function and neuropathy in the corpus cavernosum [10]. In the erection physiology, nitric oxide (NO) has been recognized as a critical molecule [11]. Studies have shown that the reduction of NO production or bioavailability depends on an increase of reactive oxygen species (ROS) and leads to impaired endothelial function [12]. In the present study, we investigated the occurrence of physiopathological changes in selected ED patients with and without type 2 diabetes mellitus

* Corresponding author at: Department of Medical Biotechnologies, University of Siena, Via Aldo Moro 2, 53100 Siena, Italy.
E-mail address: corteale@gmail.com (A. Cortelazzo).
[1] The first two authors contributed equally to the generation of this article.

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(T2DM), using routine hematology/clinical chemistry and proteomic analyses.

2. Materials and methods

2.1. Study population

A total of 247 patients presenting as main complaint ED were examined at our outpatient office from January 2014 to December 2014. Patients without disorders of thyroid and pituitary glands, and without primary hypogonadism were selected. Patients with genital abnormalities or previous urological surgery were excluded. Subjects were subdivided in three groups: (A) healthy controls (n = 60, enrolled from the outpatient activity on prevention of andrological disorders), (B) non-diabetic ED patients (n = 30) and (C) T2DM ED patients (n = 30). In order to identify if any changes are associated with DM or truly with T2DM ED, a group of diabetic patients (n = 25) was selected. In order to define if age has any role in ED patients, an old age group of non-diabetic ED patients (n = 25) was considered.

Patients were comprehensively assessed with a detailed medical history including laboratory exams. Moreover, patients were requested to complete the International Index of Erectile Function [13]. In accordance with the National Cholesterol Education Program [14], as the main emphasis is on central obesity defined by a waist circumference (WC) ≥94 cm in Caucasian population, we elected the measure of WC than body mass index. Clinical characteristics of studied groups are listed in Table 1. This study was approved by the Institutional Review Board of Azienda Ospedaliera Universitaria Senese. All patients provided their informed consent prior to inclusion in the study.

2.2. Blood sampling

Blood samples from ED patients, diabetic subjects and healthy controls were collected in heparinized tubes at 8 AM after overnight fasting, and all manipulations were carried out within 2 h after the collection. Blood samples were centrifuged at 2400 *g* for 15 min at 4 °C. The platelet poor plasma was saved and the buffy coat was removed by aspiration. Plasma samples used for proteomic analysis, contained phenylmethylsulfonyl fluoride (1 mM) as a protease inhibitor, were stored at −70 °C until use.

2.3. Routine hematology/clinical chemistry

Basal glycemia (reference range: 70–110 mg/dL), total cholesterol (reference range: 130–200 mg/dL), triglycerides (reference value: <200 mg/dL) and high-density lipoprotein (HDL, reference value: >55 mg/dL), were analyzed at University Hospital Laboratory of Clinical Pathology and were determined by absorption photometry using the Cobas 6000 system (Roche Diagnostic). Low-density lipoprotein (LDL) subsequently was calculated using the Friedewald formula [15]. Hypercholesterolemia was defined when lipid lowering therapy was taken and/or HDL was <55 mg/dL. Similarly, hypertriglyceridemia was defined when plasma triglycerides were >200 mg/dL. We elected to measure circulating total testosterone (Tt, reference range: 2.8–8 ng/mL) and Sex Hormone Binding Globuline (SHBG, reference range: 10–57 nmol/L) by IRMA using a kit from Diagnostic Systems Laboratories (DSL, Webster, Texas, USA). Laboratory characteristics of studied groups are listed in Table 1.

2.4. Two-dimensional gel electrophoresis

Two-dimensional gel electrophoresis (2-DE) analysis was performed according to Görg et al. [16] with slight modifications. Samples containing 60 μg of proteins determined with bicinchoninic acid assay (Pierce, Thermo Fisher Scientific), were combined with solubilizing buffer containing 8 M urea, 2% 3-(3-cholamidopropyl)-dimethyl-ammonio-1-propane sulfonate, 0.3% dithiothreitol (DTT), 2% immobilized pH gradient (IPG) buffer, and a trace of bromophenol blue and loaded into IPG strips 3–10 non-linear on an Ettan IPGphor (GE Healthcare, Uppsala, Sweden) apparatus system and rehydrated for 7 h. Isoelectric focusing was carried out for a total of 32 kV h. After focusing, the strips were first equilibrated with a buffer containing 50 mM Tris–HCl, pH 8.8, 6 M urea, 2% w/v SDS, 30% v/v glycerol, and 1% w/v DTT for 15 min; then they were equilibrated again with the same buffer described above, except it contained 4% w/v iodoacetamide instead of DTT and a trace of bromophenol blue. The strips were washed further for 10 min with Tris–glycine buffer. The second dimension was performed on an EttanDalt Six Electrophoresis system (GE Healthcare, Uppsala, Sweden). IPG strips and a molecular weight (MW) standard were embedded at the top of a 1.5 mm thick vertical polyacrylamide gradient gel (8–16% T) using 0.5% w/v agarose and run at a constant current of 40 mA/gel at 20 °C. Each sample was carried out in triplicate under the same conditions.

2.5. Western blotting for 4-hydroxy nonenal protein adducts

After 2-DE, proteins (80 μg of total protein), were electro-transferred (0.8 mA/cm^2; 1 h, 40 min) onto a hybond enhanced chemiluminescence nitrocellulose membranes (Bio-Rad, Milan, Italy) using a Pharmacia Biotech Nova Blot semi-dry transfer instrument (GE Healthcare Europe Gmbh, Milan, Italy). Membranes were then blocked for 1 h in Tris-buffered saline, pH 7.5, containing 0.5% Tween 20 (TBST) and 3% nonfat dry milk, blotting-grade blocker (Bio-Rad, Milan, Italy). Membranes were incubated overnight at 4 °C with the primary antibody, goat anti 4-hydroxy nonenal (4-HNE) (1:1000) (Millipore Corporation, Billerica,

Table 1
Clinical and laboratory characteristics of studied groups.

Variables	Healthy controls	Diabetic patients	Non-diabetic ED patients		Diabetic ED patients
	n = 30	n = 25	n = 30	n = 25	n = 30
Age	50.5 (46.5–53.25)	67 (56–73.25)	47 (41–55.5)	64 (55.5–73)	69 (55.5–75)
WC, cm	89.5 (86–93.5)	98 (95.5–107.25)	94 (89.25–101.25)	98.5 (91.5–102)	101 (95–104.25)
Basal glycemia, mg/dL	79.5 (74.25–84.75)	140 (137.25–144.25)	87.5 (81.25–95.25)	92 (86.5–96.75)	135 (132.5–137.5)
Total cholesterol, mg/dL	182 (163.5–206.25)	193 (174.5–215.25)	210 (168.75–241.25)	216 (170.5–250)	225 (206–257.5)
Triglycerides, mg/dL	147 (131–161)	152 (139–166)	183 (164–192.75)	175 (155–186)	170 (156–179)
LDL, mg/dL	102 (86–126)	123 (90–134)	147 (137–180)	152 (130–181)	160 (137–188)
HDL, mg/dL	48 (46.75–50)	46 (44.5–47.25)	36 (35.5–37.5)	39 (37.75–40)	40 (38.5–41)
Tt, ng/mL	4.2 (3.6–4.6)	3.79 (3.1–4.3)	3.9 (2.9–4.2)	3.81 (3.2–3.9)	3.75 (3.3–3.8)
SHBG, nmol/L	35.7 (30.2–42.9)	43.8 (35.4–51.5)	44.2 (39.3–56.6)	51.3 (40.6–58.5)	56.4 (35.7–62.3)

Clinical and laboratory characteristics of the population were compared using ANOVA. Median values are reported with 95% confidence intervals in brackets.