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Randomized Control Trials

Effects of metformin on markers of oxidative stress and antioxidant reserve in patients with newly diagnosed type 2 diabetes: A randomized clinical trial

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SUMMARY

Background & aims: Given the long term benefits observed with metformin use in diabetes patients, a role in modulating oxidative stress is imputable. Effects of metformin on markers of oxidative stress, antioxidant reserve, and HDL-c associated antioxidant enzymes were investigated.

Methods: In a clinical trial setting (Registered under Clinical Trials.gov Identifier no. NCT01521624) 99 medication-naïve, newly diagnosed type 2 diabetes patients were randomly assigned to either metformin or lifestyle modification. AOPP, AGE, FRAP, activities of LCAT, and PON were measured at baseline and after 12-weeks.

Results: Baseline values of the oxidative stress markers did not differ significantly between the two groups. In cases, after three months treatment, there was a significant reduction in AOPP (137.52 ± 25.59, 118.45 ± 38.42, $p < 0.001$), and AGE (69.28 ± 4.58, 64.31 ± 8.64, $p = 0.002$). FRAP and PON increased significantly (1060.67 ± 226.69, 1347.80 ± 251.40, $p < 0.001$ and 29.85 ± 23.18, 37.86 ± 27.60, $p = 0.012$ respectively). LCAT levels remained unchanged (45.23 ± 4.95, 46.15 ± 6.28, $p = 0.439$). Comparing the two groups in a final multivariate model, AOPP, FRAP, and AGE levels changed more significantly in metformin compared with lifestyle modification alone ($p = 0.007$, $p < 0.001$ and $p < 0.001$ respectively). Escalation in LCAT or PON activities did not differ between the two groups ($p = 0.199$ and 0.843 respectively).

Conclusions: Use of metformin is more effective in reducing oxidative stress compared with lifestyle modification alone.

1. Introduction

Sedentary lifestyle and constant over-nutrition in the past decades, have led to a disproportionate growth in type 2 diabetes; making this silent yet debilitating disease a major health predicament in the 21st century.[1] With ever growing figures, it is projected that by 2030, the total number of people with diabetes will rise to 552 million.[2] It is estimated that in 2007 the United States alone, spent $174 billion for diabetes of which $58 billion were attributable to diabetes-related chronic complications.[3]

Oxidative stress plays a key role in the pathogenesis of diabetes complications, including vascular disease, nephropathy, retinopathy, and even neuropathy associated with diabetes.[4–7] Oxidative stress is defined as the state of increased reactive oxygen species and/or impaired inherent anti-oxidant support. Chronic hyperglycemia and disturbed lipid regulation commonly seen in diabetes are the main sources of this process.[8,9] Despite the critical role of oxidative stress in diabetes, most clinical trials with available antioxidants and vitamins have either failed to show any long term benefits or have produced inconsistent results.[10,11] Hence, there has been growing interest in elucidating the possible roles of oral hypoglycemic agents including metformin in reduction of oxidative stress. Metformin, the most common prescribed oral medication in

Abbreviations: AOPP, advanced oxidation protein products; AGE, advanced glycation end products; FRAP, ferritin reducing ability of plasma; LCAT, lecithin cholesterol asyltransferase; PON, paraoxonase.

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type 2 diabetes, lowers HbA1c around 1.5%, rarely causes hypoglycemia (compared with insulin or sulfonylureas), has relatively few contraindications, its adverse effects are generally tolerable, does not cause weight gain, is cheap, and is highly acceptable among patients.[12] Metformin exerts its main antihyperglycemic effects through activation of AMP-activated protein kinase, resulting in reduced hepatic gluconeogenesis.[13] In addition, moderate improvements in lipid profile and weight reduction have been reported with metformin use.[13] In the UKPDS study, metformin has been shown to lower the risk of diabetes related complications, cardiovascular disease, stroke, and all-cause-mortality.[14] Recently, the REACH study demonstrated that even in a subset of patients that metformin is not currently recommended (i.e. congestive heart failure and chronic kidney disease) metformin decreases mortality.[15]

Given the long term benefits observed with metformin use, a role in modulating oxidative stress is imputable.[16,17] We designed this study to evaluate the actions of metformin on oxidative stress in a group of medication-naïve newly diagnosed type 2 diabetes patients. Advanced oxidation protein products (AOPP) and advanced glycation end products (AGEs) were measured as markers of oxidative stress. Ferritin reducing ability of plasma (FRAP) was also measured to represent inherent antioxidant capacity of plasma. Additionally, since HDL-c associated enzymes, namely paraoxonase (PON) and lecithin cholesterol asyltransferase (LCAT) exhibit keen anti-oxidant properties; we postulated that their activity might be influenced by metformin as well.

2. Patients and methods

2.1. Design

A single-center, open label randomized clinical trial was conducted. Patients were recruited through diabetes clinic of Vali-Asr hospital (Tehran, Iran) from October 2010 to March 2011. Subjects were found eligible if the following criteria were met: (1) diagnosis of type 2 diabetes mellitus based on American Diabetes Association (ADA) criteria; (2) No history of serious chronic illnesses of heart, lung, and kidney; (3) No prior treatment with anti-diabetes medications for either diabetes or conditions associated with hyperglycemia; (4) No intake of prescribed or over-the-counter vitamins C and E in the past year; (5) No intake of aspirin in the past year; (6) No history of alcohol intake in the past month; (7) No history of current or past smoking. First subject was allocated to the case group and the next was recruited as control; this process was continued until each group consisted of 52 subjects. Case group received 1000 mg metformin daily plus advice for lifestyle modification while control group only received consultation for changing lifestyle. For lifestyle modification, subjects were instructed regarding (1) benefits of gradual moderate weight loss (7% of body weight) in control of hyperglycemia; (2) advantages of a balanced diet (limited use of simple carbohydrates, saturated fatty acids, increased intake of whole grains and dietary fibers with overseeing total calorie intake); and (3) necessity of a regular exercise plan of moderate intensity (50–70% of maximum heart rate) for at least 30 min, five times a week; and if no contraindications exist, resistance training for three times a week. Adherence to these recommendations was not officially assessed during the follow-up time. Those receiving metformin were asked to return if they experienced any significant adverse effects (e.g. severe gastrointestinal discomfort). After 3 months, subjects returned for a scheduled follow-up visit and were interviewed and examined using the same protocol as baseline (see below).

Written informed consent was obtained from each subject regarding confidentiality and anonymity of data collected, but details and purpose of the study were not disclosed. Tehran University of Medical Sciences board of ethics approved the study protocol.

2.2. Assessment

During the initial visit, patients were interviewed according to a pre-designed questionnaire and underwent a thorough physical examination afterward. Blood pressure was measured using a standard sphygmomanometer (Riester, Big Ben adults, Germany). Subjects were asked to rest in a sitting position for at least 10 min; two readings with 5-min intervals were averaged. Mean arterial pressure (MAP) was calculated as follows: diastolic pressure times two, added to systolic pressure, and then divided by three. Employing standard stadiometer, height was measured with subjects standing; the nearest 0.1 cm was recorded. Weight was assessed with a digital scale (Beurer, GS49, Germany), and only light clothing was allowed. Body mass index (BMI) was calculated using the Quetelet formula of weight in kilograms divided by height squared in meters (kg/m^2). Waist circumference was measured at mid-line between lower costal margin and iliac crest using an inflexible measurement tape. In a similar fashion, hip circumference was assessed at a point where hip is widest. Readings were recorded with an accuracy of 0.1 cm. Same examinations were performed at the 3-month follow-up visit.

2.3. Laboratory evaluations

Subjects were instructed to go on an overnight fasting for at least 10 h in both initial and 3-month follow-up visits. The next morning, 10 mL of venous blood sample was drawn in the hospital laboratory. Fasting plasma glucose (FPG) concentrations were assessed by enzymatic calorimetric method using glucose oxidase (GOD) test. Radioimmunoassay techniques (Immunotech, Prague, Czech Republic) were employed to determine concentrations of fasting serum insulin. Percentage of glycated hemoglobin A1c (HbA1c) was determined using high performance liquid chromatography (HPLC). Enzymatic methods (Pars Azmun commercial kits, Karaj, Iran) were employed to measure serum concentrations of total cholesterol, high density lipoprotein cholesterol (HDL-c), low density lipoprotein cholesterol (LDL-c), and triglycerides. Quantitative measurement of serum highly sensitive C-reactive protein (hsCRP) was done with commercial kits (Diagnotics Biochem Canada Inc, Canada) using ELISA. Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated according to the formula of Matthews et al. (1985): Fasting insulin (IU) multiplied by FPG (mg/dL), divided by 405.[18]

2.3.1. AOPP

AOPP concentrations were determined with spectrophotometric methods (FLUOstar OPTIMA, BMG, Germany) as described by Kalousova et al. (2002).[19] In this method, 200 μL of serum is diluted by a factor of 5, in phosphate buffered saline (PBS). In addition, 200 μL of chloramin T (0–100 μmol/l) for calibration, and 200 μl of PBS as blank is also added to different microplates. Finally, 10 μL of acetic acid, and 20 μL of 1.16 M potassium Iodide (KI) is added to preparations. Measurements are done at absorbance of 340 nm and are expressed in μmol/L.

2.3.2. FRAP

FRAP was measured with spectrophotometry as described by Benzie and Strain (1996).[20] Based on this method, FRAP reagent is prepared with mixing 300 mmol/L of acetate buffer (pH: 3.6), 10 mmol/L of tripyridyl triazine (TPTZ) in 40 mmol/L HCL, and 20 mmol/L $FeCl_3.6H_2O$. Twenty five μL of serum is then added to