Complementary Therapies in Clinical Practice 19 (2013) 97–100

Contents lists available at SciVerse ScienceDirect

Complementary Therapies in Clinical Practice

journal homepage: www.elsevier.com/locate/ctcp

Effect of daily supplementation of fruits on oxidative stress indices and glycaemic status in type 2 diabetes mellitus

Shreelaxmi V. Hegde [a,*], Prabha Adhikari [b], Nandini M [c], Vivian D'Souza [c]

[a] Department of Biochemistry, Srinivas Institute of Medical Science and Research Centre, Mukka, Mangalore 574021, Karnataka, India
[b] Department of Medicine, Kasturba Medical College and Hospital, Manipal University, Mangalore, Karnataka, India
[c] Department of Biochemistry, Kasturba Medical College and Hospital, Manipal University, Mangalore, Karnataka, India

Keywords:
Oxidative stress
Antioxidants
Diet therapy
Glycated haemoglobin
Type 2 diabetes mellitus

A B S T R A C T

This study sought to examine whether consumption of two low-calorie fruit/day for 3-months can effectively improve oxidative stress, anthropometry, blood pressure and glycaemic control in type 2 diabetes mellitus. Study involved 123 patients who were assigned to receive either standard care or with additional dietary therapy. Dietary intervention resulted in significant reduction in malondialdehyde, plasma glucose, glycated haemoglobin and improvement in antioxidants like vitamin C and reduced glutathione when compared to controls. Mean plasma levels of vitamin C increased by 64% ($p < 0.001$). There were no differences in waist circumference, waist-to-hip ratio, blood pressure, vitamin E and superoxide dismutase in the intervention group at follow-up. Diet rich in fruits can improve some antioxidants which are likely to reduce oxidative stress in type 2 diabetes. Regular consumption of fruits can lower the glycaemic status in these patients. The study supports the usefulness of plasma vitamin C as a biomarker for fruit intake.

1. Introduction

The worldwide burden of type 2 diabetes is increasing rapidly and is projected to be atleast 366 million by the year 2030.[1] One of the pathogenic mechanisms that increase the risk for diabetes and its complications is the imbalance between pro-oxidants and antioxidants, called as oxidative stress (OST).[2] Hyperglycemia induced nonenzymatic glycosylation, polyol pathway, glucose auto-oxidation and monocyte dysfunction leads to continuous production of free radicals. One way of combating this increased OST would be to increase antioxidant defences. This can be achieved either by increase in dietary intake of fruits and vegetables or consumption of antioxidant supplements. Epidemiological evidence demonstrate the benefits of a diet rich in fruits, vegetables and whole grain cereals in diabetes[3–6] but the results are not entirely consistent. In many of the studies dietary antioxidant intake is calculated by self-reported diet questionnaires. The major drawback of food frequency questionnaires (FFQ) is that it may overestimate the fruit and vegetable consumption.[7] This may be one of the reasons for the inconsistent associations between fruit and vegetable consumption and diabetes risk reported earlier.[3–6] Furthermore, intake of fruit juices are positively associated with incidence of type 2 diabetes, whereas consumption of whole fruit and vegetables are inversely associated.[8] Whole fruits may contribute to a decreased incidence of type 2 diabetes through their low energy density, low glycaemic load and high fibre and micronutrient content.[9] With this background in the present study we aimed to estimate the effect of 3-months dietary antioxidant intake on the levels of OST, antioxidant status, glycaemic level, anthropometry and blood pressure in type 2 diabetes mellitus.

2. Materials and methods

The participants were recruited from the outpatient diabetes clinic of Kasturba Medical College Hospital (KMCH), Mangalore. A written informed consent approved by the institutional ethical committee was collected at the beginning of the study. This study comprised of 123 type 2 diabetes patients between the ages 40–75 years, non-smokers, non-alcoholics and with no history of acute macrovascular complications, cancer, pulmonary tuberculosis or any serious systemic illness. Stratified sampling was adopted at the time of allocation to groups. This was to maintain an equal number of patients with uncomplicated diabetes and with microvascular complications, macrovascular complications and peripheral neuropathy in these groups. Subjects were grouped as 60 for diet intervention and 63 as controls. Dietary counselling was conducted by registered dieticians at the start of the study to know the pattern of daily food intake by these patients. It was observed that these

* Corresponding author. Tel.: +91 824 2425966, +91 9986668611 (mobile); fax: +91 824 2442766.
E-mail address: shreelaxmi.hegde@gmail.com (S.V. Hegde).

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http://dx.doi.org/10.1016/j.ctcp.2012.12.002

patients consumed 1 or less servings of fruit on daily basis. They had the fear that fruit consumption could increase their blood glucose level. Subjects in the intervention group were suggested to increase their antioxidant intake by having 2 fruit (medium sized) per day for 3-months other than their regular diet. Fruits were chosen such that they were low calorie and available at all seasons of the year. Fruits included- 1 sweet lime/1 orange/1 apple/10 slices of papaya. Subjects were made to maintain a diet diary and record the daily intake of these fruits. The control group maintained their usual eating habits. No recommendation was made about the use of vitamin supplements. Drug dosages with regard to diabetes and blood pressure were kept constant throughout the study period.

During the intervention period the dietician scheduled intermittent telephone calls to maintain enthusiasm and adherence to the antioxidant diet among the subjects. The patient's family members were also enquired to know the patients proper compliance. The subjects in the control group were also enquired about their diet and drug dosages. All patients maintained physician visits at a monthly interval.

2.1. Clinical studies

At baseline and after 3-months, all study subjects completed a medical-history questionnaire and underwent a physical examination that included body mass index (BMI), waist circumference, waist-to-hip ratio and blood pressure (BP) measurements. Weight was measured while the subjects were minimally clothed without shoes using digital scales and recorded to the nearest 0.1 kg. Height was measured in a standing position without shoes using a stadiometer. Waist-to-hip ratio was calculated as waist circumference in centimetres divided by hip circumference in centimetres. Waist circumference was measured using a non-stretchable inch tape positioned midway between iliac crest and lower rib cage with the measurement taken at the end of expiration while the patient is breathing quietly. Blood pressure was recorded by taking mean of second and third readings of blood pressure taken 5 min apart in sitting position after the patient had completely relaxed.

2.2. Biochemical assessments

Plasma glucose concentration was analysed by clinical routine enzymatic techniques. Glycated haemoglobin (HbA_1c) was analysed by particle enhanced immunoturbidimetric method using Dia Sys diagnostic kits, Holzheim, Germany. Oxidative stress status was evaluated by measuring the blood levels of malondialdehyde (MDA), a product of lipid peroxidation and individual antioxidants. Malondialdehyde and reduced glutathione (GSH) were assayed in the RBC's by the method of Stocks and Dormandy[10] and Beutler et al. method.[11] Vitamin C and E in the plasma were measured by 2,4 dinitro phenyl hydrazine[12] and Bieri et al. methods.[13] Superoxide dismutase (SOD) in RBC's was analysed by Beauchamp and Fridovich method.[14]

2.3. Statistical methods

Data were analysed using SPSS software (version 11.0). Paired '*t*' test was used to compare the continuous variables from baseline to follow-up. Mann–Whitney *U* test, a non-parametric test was used to compare the differences in various parameters before and after intervention between the two groups. Data are expressed as mean ± S.D. where appropriate.

3. Results

One twenty one patients completed the study. Out of the 2 dropouts from the intervention group one moved and one withdrew from the study. Out of 58 subjects who completed the dietary intervention, 51 participants consumed two fruits on all days of the study. Remaining three subjects consumed fruits on an average of 4 days/week, two subjects for less than twice/week and two participants did not eat any fruits. With the intention-to-treat, all were included in the final analysis.

The mean (SD) age was 58.5 (9.6) in the intervention group and 57.5 (8.9) in the control group. There were no significant differences between the two groups with regard to age, sex and duration of diabetes or hypertension. At baseline there were no differences between the groups in any of the study variables.

The changes in mean blood levels of various antioxidants and MDA are shown in Table 1. We observed a drastic increase in vitamin C over baseline by 64% ($p < 0.001$) in the intervention group. Control group showed 20% reduction in the vitamin C levels. The study also revealed a significant reduction in reduced GSH (11%) level in the control arm (<0.05) when compared to the intervention group. Other antioxidants like vitamin E and SOD

Table 1
Changes in selected clinical and oxidative stress variables from baseline to the end of 3 month follow-up in the intervention and control groups.

Variables	Intervention group ($n = 60$)			Control group ($n = 63$)			p Value[a]
	Baseline	After 3 months	Change at 3 months	Baseline	After 3 months	Change at 3 months	
Fasting plasma glucose (mmol/l)	7.9 ± 1.5	7.2 ± 1.6	−0.7 ± 0.1	8.6 ± 3.1	9.0 ± 3.0	0.4 ± 0.1	<0.001
Post prandial plasma glucose (mmol/l)	11.6 ± 3.2	10.0 ± 2.7	−1.6 ± 0.5	12.3 ± 5.1	12.6 ± 4.7	0.3 ± 0.4	<0.001
HbA_1c (%)	8.0 ± 1.3	7.7 ± 1.3	−0.3 ± 0.0	8.0 ± 1.5	8.5 ± 1.8	0.5 ± 0.3	<0.001
BMI (kg/m^2)	24.4 ± 3.9	24.5 ± 4.0	0.1 ± 0.1	25.3 ± 3.9	25.5 ± 4.1	0.3 ± 0.2	0.309
Waist circumference (cm)	88.7 ± 10.2	88.7 ± 9.8	0.0 ± 0.4	90.5 ± 9.8	90.0 ± 9.1	−0.5 ± 0.7	0.424
Waist-to-hip ratio	0.92 ± 0.06	0.92 ± 0.05	0.0 ± 0.01	0.93 ± 0.06	0.93 ± 0.05	0.0 ± 0.01	0.011
Systolic blood pressure (mmHg)	140.2 ± 20.6	135.0 ± 16.6	−5.2 ± 4.0	139.6 ± 21.0	138.0 ± 15.4	−1.6 ± 5.6	0.099
Diastolic blood pressure (mmHg)	83.5 ± 9.8	81.8 ± 9.0	−1.7 ± 0.8	84.1 ± 9.9	83.9 ± 8.5	−0.2 ± 1.4	0.313
Malondialdehyde (μmol/L)	50.2 ± 9.3	47.4 ± 9.4	−2.8 ± 0.1	50.7 ± 9.3	52.3 ± 10.9	1.6 ± 1.6	<0.001
Glutathione (μmol/gmHb)	6.5 ± 2.4	6.7 ± 2.4	0.2 ± 0.0	7.1 ± 3.6	6.3 ± 2.4	−0.8 ± 1.2	<0.05
Vitamin C (μmol/L)	22.1 ± 13.6	36.3 ± 22.7	4.2 ± 9.1	30.0 ± 29.0	23.8 ± 18.2	−6.2 ± 10.8	<0.001
Vitamin E (μmol/L)	47.8 ± 15.6	46.7 ± 14.4	−1.1 ± 1.2	59.7 ± 24.2	58.0 ± 23.5	−1.7 ± 0.7	0.338
Superoxide dismutase (unit/gmHb)	5390.0 ± 1116.0	4920.0 ± 971.0	−470.0 ± 145.0	5691.6 ± 1641.4	5593.1 ± 1768.0	−98.5 ± 126.6	0.066

Values are mean ± SD.
[a] p Values are significance values in intervention group when compared to the control group.