High levels of F2-Isoprostanes in Jamaican adults with diabetes mellitus

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Abstract

Inadequate glycaemic control in Jamaican adults with type 2 diabetes mellitus (DM) prompted assessment of glycaemic control, oxidative stress, cardiovascular (CV) and renal risk in adults attending a hospital clinic. A random sample of 133 patients men (n=35) and women (n=98), with diabetes mellitus was selected from a population of 510 patients. Fasting blood samples (n=122) were evaluated for metabolic control and dyslipidaemia. Oxidative stress was evaluated by measurement of urinary F_2 isoprostane (n=124). The data were analysed using SPSS. The mean age of the participants was 56.7 ± 14.3 years, with mean duration of diabetes of 12.2 years. Mean fasting blood sugar was 8.6 ± 4.3 mmol/L. 77% of patients had HbA_{1c} > 6.5%. 69% of patients were being treated with insulin with no difference in HbA_{1c} levels in these patients compared to those receiving other hypoglycaemic agents. 90% of men (median 1004pg/mg creatinine) and 99% of women (median 1501.3pg/mg) had isoprostane levels above the median for subjects with CV risk. 54% of patients had total cholesterol levels ≥ 5.2 mmol/L, 16% triglyceride levels ≥ 1.5 mmol/L, 25% HDL levels ≤ 1.0mmol/L and 86% LDL ≥ 2.5mmol/L. 63 % of patients had BP >130/85 mmHg. 81% were overweight or obese, with 80% of the men having waist circumferences >88 cm, whereas 87% of the women had waist circumferences of 84.5cm. Microalbuminuria was increased in 37% of the subjects. The high prevalence of overweight, central obesity, elevated LDL and hypertension in these patients indicated high CV and renal risk. The risk was significantly higher in persons with inadequate glycaemic control. Isoprostane levels were high in the majority of subjects but did not correlate with HbA_{1c} or any other variable. (Int J Diabetes 14: 51-54, 2006)

Key words: *Isoprostanes, cardiovascular risk, renal risk, BMI*

Introduction

Hyperglycaemia increases the risks for the chronic complications of diabetes and thus increases morbidity and mortality. Improved glycaemic control may prevent the appearance and enhance the regression of macrovascular and microvascular complications. Macrovascular complications make the greatest contribution to the burden of diabetes[1] and are decreased with glycaemic control.[2] The prevalence of DM is high in Jamaica and the Caribbean and many patients have poor metabolic control.[3,4]

Heart disease is the leading cause of death in persons with DM.[5] Cardiovascular (CV) disease in diabetes is multifactorial and risk factors include hypertension, dyslipidemia, insulin resistance and central obesity.[6] Recently, oxidative stress has been suggested as another potential mechanism. Possible sources of oxidative stress in DM include altered carbohydrate and lipid metabolism and decreased levels of antioxidant defenses. Investigation of oxidative stress may therefore be useful in understanding DM and CV disease. Growing evidence suggests that isoprostanes are reliable and sensitive markers of *in vivo* lipid peroxidation. Studies have shown an effect of glucose on oxidative stress, and positive associations between indices of obesity such as BMI and waist/hip ratio and urinary levels of 8-epi-PGF_{2a}. Insulin itself promotes hydrogen peroxide generation in fat cells, prompting speculation that oxidative stress is a mechanism of insulin resistance in chronic hyperinsulinaemia.[7] A previous study on subjects with at least one CV risk factor but no history of CV disease, reported a median (range) of 141 (67-498) pg/mg creatinine in subjects treated with vitamin E supplementation and 148 (76-561) pg/mg creatinine in untreated subjects.[8] Another study reported high values in subjects with android obesity.[9]

Hypertension is known to be a major risk factor for the development of diabetic renal disease and hyperglycaemia also has a role in the development of diabetic nephropathy.[10] Microalbuminuria is a predictor of CD disease and renal failure in populations of European origin and in other races such as Australian Aborigines.[11] This study therefore sought to assess the level of glycaemic control and indicators for CV and renal risk, including oxidative stress, in adults attending a hospital clinic in Jamaica.

Methods

This study was approved by the Faculty of Medical Sciences/University Hospital of the West Indies Ethics Committee. A random sample of 133 patients, 35 men and 98 women with DM was selected from a population of 510 patients attending the University Hospital of the West Indies (UHWI) Specialist clinic.

Anthropometric data were obtained by measuring height, weight, and waist circumference. A mean of two blood pressures (BP) was also taken. Urine (n=124) and fasting

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Table1: Participant's mean values for $HbA_{1c,}$ fasting blood sugar, serum lipids and microalbuminuria with suggested target values

Variable	Mean ± SD	Target Values
HbA_{1c} %	8.7± 2.4	≤ 6.5
Fasting blood sugar (mmol/L)	8.6 ± 4.3	≤ 6.7
Total cholesterol (mmol/L)	5.4 ± 1.1	≤ 5.2
HDL (mmol/L)	1.2 ± 0.3	> 1.0
LDL (mmol/L)	3.6 ± 1.0	≤ 2.5
Microalbumin (mg/L)	74.0	<30.0

LDL= low density lipoprotein, HDL= high density lipoprotein

Table 2: Percentage of Population with CVD risk indicators.

Risk Indicators	% at risk
LDL > 2.5 mmol/L	86.1
TGs > 1.5 mmol/L	16.3
SBP > 130 mmHg	63.0
BMI > 25 kg/m^2	81.0
HbA_{1c} > 6.5%	77.0
Microalbumin > 30mg/L	37.0

LDL= low density lipoprotein, TGs = triglycerides, SBP= systolic blood pressure, BMI= body mass index

Table 3: Comparison of duration of DM, microalbumin and lipid status in well controlled and poorly controlled patients.

Variable	HbA_{1c} ≤ 6.5%	HbA_{1c} > 6.5%	*P*-value
Duration of DM (years)	7.43 ± 8.72	13.38 ± 9.10	0.003
LDL (mmol/L)	3.25 ± 0.82	3.73 ± 1.06	0.041
VLDL (mmol/L)	0.40 ± 0.19	0.54 ± 0.34	0.039
Triglycerides (mmol/L)	0.86 ± 0.40	1.18 ± 0.74	0.039
HDL (mmol/L)	1.37 ± 0.33	1.19 ± 0.29	0.008
Microalbumin (mg/L)	15.54 ± 22.44	93.79 ± 18.92	0.001

t-test
LDL = low density lipoprotein, VLDL = very low density lipoprotein, HDL= high density lipoprotein.

blood samples (n=122) were obtained for clinical tests. Urine samples were stored at -20^{o}C, 1ml of whole blood was stored at -70^{o}C for HbA_{1c} analysis. All other blood samples were spun at 1000g for 10 min to obtain sera and plasma that were stored in micro-centrifuge tubes at -70^{o}C until analysis. HbA_{1c} and microalbumin measurements were carried out using the DCA 2000 analyzer (Bayer). Serum lipids were measured using the Abbot Autoanalyzer. Urinary 15 isoprostane F_{2t} was determined by competitive enzyme-linked immunoassay (ELISA) using commercial immunoassay kits obtained from Oxford Biomedical (UK).

The values used to determine risk indicators were: LDL >2.5mmol/L, triglyceride levels >1.5mmol/L, systolic BP >130mm Hg, BMI >30 kg/m^2, HbA_{1c} >6.5%, microalbumin excretion >30mg/L. The cut off points for waist measurements were 88cm for men and 84.5cm for women.[12] Isoprostane values were compared with those of a previous study on subjects with cardiovascular risk.[8]

Data analysis: Spearman's Correlation Coefficient was used to determine any associations between the variables. Mann-Whitney-*U* tests and *t*-tests were used to compare groups. The Statistical Package for the Social Sciences, Version 10.0 was used.

Results

Mean age was 56.7 ± 14.3 years, with mean time since diagnosis of 12.2 years. Men were significantly older (Z = -2.6, P = 0.008) than the women. The majority of the participants (81%) were overweight or obese, with a BMI range from 16.6-47.4 kg/m^2. 45% and 22.9% of the men were overweight and obese respectively compared to a significantly higher proportion of women (Z =-3.5, P = 0.001) with 34.7% and 45.9% respectively, using the WHO criteria. Central obesity was common: 80 % of the men had waist circumferences >88cm and 87% of the women had circumferences >84.5cm. Waist circumferences were associated with BMI (r=0.85, P=0.02). BMI correlated with VLDL levels (r=0.21, P=0.02). 63% of patients had systolic BP >130 mmHg. Mean fasting blood sugar was 8.6 ± 4.3 mmol/L with 58% having values ≥ 6.7mmol/L. Only 23% had HbA_{1c} ≤ 6.5%. 54% of patients had total cholesterol levels ≥ 5.2 mmol/L, 16% had triglyceride levels ≥1.5 mmol/L, 25% had HDL cholesterol levels ≤ 1.0 mmol/L and 86% had LDL cholesterol ≥ 2.5mmol/L. 12% of patients reported being on lipid-lowering drugs. Women had higher HDL values than men (Z = -3.0, P = 0.003). Microalbumin excretion was elevated in 37% of the subjects (Tables 1, 2).

The median isoprostane level in men was 1004.6 pg/mg creatinine, quartiles: 515.9 to 2077.8 pg/mg, range 80.6 to 6425.4 pg/mg. In women, the median level was 1501.3 pg/mg creatinine, quartiles: 852.8 to 2138.2 pg/mg and range: 50.74 to 11464.4 pg/mg creatinine. The differences between the values for men and women were not significant. The majority (90%) of men were above the median for subjects at CV risk, 148 pg/mg creatinine, 80% were above the upper limit of the range, 561 pg/mg creatinine. 99% of women were above the median for subjects at CV risk and 90% were above the upper limit of the range.[8] The individual with the highest isoprostane value, 11464 pg/mg creatinine, had rheumatoid arthritis, LDL 2.69mmol/L and BMI 31.2 kg/m^2. The lowest value, 50.7 pg/mg creatinine was found in another female patient who had normal BMI, two years duration of disease, normal LDL and HBA_{1c} who regularly used a multivitamin

supplement high in antioxidants. Although 26% of participants reported taking antioxidants such as Vitamin E, no effect on isoprostane levels could be demonstrated.

The majority (77%) had poor glycaemic control. Significant differences were found in duration of diabetes, LDL, VLDL, triglycerides, HDL and microalbuminuria, between those who were poorly controlled (HbA_{1c} >6.5%) and those who were well controlled ($HbA_{1c} \leq 6.5\%$), but there were no differences in the isoprostane values (Table 3). HbA_{1c} % correlated with LDL levels (r=0.26, P=0.006).

85% of patients had two or more CV risk factors. 69% were being treated with insulin and there was no difference in HbA1c levels in these patients compared to those receiving oral hypoglycaemic agents only.

Discussion

This study is one of a few Caribbean studies to determine glycaemic control, using HbA_{1c}, in a diabetes clinic. The female/male ratio (3:1) in the sample is consistent with the population ratios found in other DM studies in Jamaica.[3] The results reveal high CV and renal risk indicators in the patients, with the majority having two or more CV risk factors. The majority of patients (77%) did not achieve glycaemic control ($HbA_{1c} \leq 6.5\%$) and therefore, as defined by the EPIC-Norfolk study, are at an increased risk for death.[13] A study in a clinic in Trinidad had concluded that poorly controlled patients had similar CV risk to better controlled patients.[4] However, our study showed significant differences in CV and renal risk indicators between those with good glycaemic control and those with poor control.

In addition to hyperglycaemia, dyslipidaemia is an important risk factor for macrovascular complications. Our results showed 86% of patients with elevated LDL and only 25% with decreased HDL cholesterol. This lipid pattern is not uncommon in African Americans. However, it has been recognized in diabetics, particularly those treated with insulin, that HDL levels need not be decreased for high CV risk.[14]

Hypertriglyceridaemia was also rare in this population, with only 4% and 15% of subjects having a triglyceride concentration in the high and borderline risk categories respectively. It is suggested that African Americans may be resistant to hypertriglyceridaemia and our subjects were of similar African origin.[15]

Hypertension is a major risk factor for developing microangiopathy and nephropathy in patients with type 2 diabetes. Lowering blood pressure is efficient at reducing the risk of progression to overt diabetic nephropathy and macrovascular complications.[10]

In this population, 63% of the participants were hypertensive and the majority had systolic hypertension.[16]

Obesity is a known independent CV risk factor and predisposes the individual to other CV risk factors. The majority of patients were overweight or obese, especially the women, and abdominal obesity was very prevalent. Waist circumference strongly correlated with BMI, supporting the suggestion that waist circumference may be used as an alternative to BMI in assessing CV risk.[12] It has been shown that for the same BMI, central obesity is associated with a far higher risk of developing concomitant disease than lower body obesity.[17,18] In this study, BMI was also associated with levels of VLDL. Microalbuminuria, a predictor of a higher CV and renal risk, was elevated in more than a third of the patients. 11% of patients had proteinuria using the urine dipstick. Simon et al[18] had found significant proteinuria in 14% of the patients in the same population. Serum creatinine was also elevated in 10% of the patients attending this clinic.

Studies of isoprostanes, in mainly Caucasian subjects, have found differences between diabetic and non-diabetic subjects. For example Italian studies reported significantly higher levels of isoprostanes in type 2 DM compared to age-matched healthy subjects.[7, 19]

In this study, urinary isoprostanes did not correlate with CV or renal risk factors and there were no significant differences in F_2 isoprostane levels between well controlled and poorly controlled diabetic subjects. The majority had isoprostane values exceeding other subjects with CV risk factors.[8] Apart from the glycaemic state, each of several factors such as obesity, dyslipidaemia, high CV and renal risk factors could have contributed to the high values of isoprostanes observed.[9] The highest value, however, could be explained by the presence of rheumatoid arthritis in the patient.[20] It has been reported that smoking leads to significantly higher levels of serum and urinary isoprostanes, but as only 5% of our participants smoked, this was not assessed as a risk factor in this population.[21, 22]

In conclusion, this study showed that a high proportion of the patients attending the clinic not only had high levels of oxidative stress, but were at increased risk for CV and renal events. Cardiovascular and renal risk indicators were significantly higher in those persons with poor glycaemic control. Improved glycaemic control must be addressed and greater attention should be placed on the management of hypertension, dyslipidaemia, obesity and oxidative stress in this population.

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