Ethnicity variation of oxidant-antioxidant enzyme activities in elderly Malaysian diabetic patients with macrovascular complications

Indran Mathavan[1], Umah Rani Kuppusamy[1], Rokiah Pendek[2], SP Chan[2] and Gracie Ong[3] *[1]Department of Allied Health Sciences, [2]Department of Medicine, [3]Department of Anesthesiology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia. (Correspondence: Dr Umah Rani Kuppusamy; e-mail: umah@ummc.edu.my)*

Abstract

The present study was designed to examine the ethnicity variation, oxidative stress and the role of oxidant-antioxidant enzymes in the development of macrovascular complications in elderly Malaysian Type 2 diabetic patients.

One hundred and eighty elderly (60-80 years) patients with Type 2 diabetes mellitus (DM) of Malay, Chinese and Indian origins and with history of severe macrovascular complications were studied for various clinical and biochemical parameters. Enzymatic assays in blood samples were estimated spectrophotometrically according to known modified methods.

Type 2 DM patients of Indian origin had significantly elevated ($P < 0.05$) mean fasting blood glucose (FBG) and glycated haemoglobin (HbA_{1c}) levels. Endogenous antioxidant enzymes namely superoxide dismutase (SOD), catalase and glutathione peroxidase (GPX) were significantly decreased ($P < 0.05$) in Indian patients compared to Malay and Chinese patients. Plasma malondialdehyde (MDA), an indicator for lipid peroxidation was significantly increased ($P < 0.05$) in Indian patients compared to the other ethnic groups, and appears to be positively correlated with FBG, HbA_{1c}, duration of disease and age in all the three ethnic groups. No significant difference was found in xanthine oxidase activity between the 3 ethnic groups but there was a mild increase when compared to control subjects (mean = 0.68, SD = 0.42).

The evidence only suggests that there is a difference between the ethnic groups and the cause is unknown based on the data presented.

Key words: ethnicity, oxidant-antioxidant enzymes, diabetes mellitus, macrovascular complications, oxidative stress.

Introduction

Macrovascular complications develop more frequently in long-standing diabetes and are a primary cause of morbidity and mortality (Caprio *et al.*, 1997). People with diabetes have an increased risk of cardiovascular disease compared with non-diabetic population, but the underlying mechanism for this increase is unknown (Hamblin, 1996; Herfindal *et al.*, 2000). It is now well established that a reduction in antioxidant defences is an important macrovascular risk factor and much evidence has indicated that oxidative stress is an acknowledged pathogenic mechanism in diabetic complication (Ceriello *et al.*, 1993; Giugliano *et al.*, 1996; Bloomgarden, 1997; Daniel *et al.*, 1998; West, 2000). The prevalence of diabetes mellitus (DM) is increasing with an excess of diabetes found in elderly population of 60 years and over and varies according to geographical and ethnic groups (King & Rewel, 1993; Mykannen, 1993). With Type 2 DM now affecting about 138 million people in Asia, monitoring the oxidant status along with lipid peroxidation index could provide additional information for evaluating progress during treatment. There are three major ethnic groups in Malaysia namely Malays, Chinese and Indians. The Indians in Malaysia are mostly from South India and Sri Lanka, whereas the vast majority of the Chinese are from South of China. The Malays are from the South Western China and Malay Archipelago comprising Philippines and Indonesian islands. There have not been any detailed studies carried out in Malaysia on ethnicity variation in relation to increased oxidative stress in elderly Type 2 diabetic population with macrovascular complications. The clinical significance of these variations can be further explored and may result in better management of disease. This study was carried out on the ethnicity variation of oxidant-antioxidant enzyme activities namely superoxide dismutase, catalase, glutatbione peroxidase, xanthine oxidase and

malondialdehyde (an indicator of lipid peroxidation) in elderly (≥ 60 years) patients with macrovascular complications.

Materials and Methods

Subjects

The group with diabetes comprised patients with poorly controlled Type 2 DM with the age range of 60-80 years attending the diabetic clinics at University Malaya Medical Centre, Kuala Lumpur. All patients were classified clinically as Type 2 according to WHO study group report (1985) recommendations. All patients had diabetes for more than 10 years and had history of documented and diagnosed severe vascular complications based on their clinical records. None of the diabetic patients had microvascular complications such as diabetic nephropathy and retinopathy. Coronary artery disease was established by ECG findings, history of myocardial ischaemia and angina. Cerebral ischaemia symptoms were determined with an abnormal carotid Doppler reading. Ischaemic foot ulcers were confirmed with Doppler ultrasound stethoscope. Peripheral vascular disease was considered to be present with ankle: brachial pressure index < 0.9. Informed consent was obtained from all the participants of the study and the protocol was approved by the ethical committee of the medical centre. The control groups comprised 60 healthy and drug free volunteers aged 46.8 ± 11.3 years and different ethnic groups (Malay = 20, Chinese = 25, Indian = 15) to match the patients with Type 2 DM. Since we failed to get enough disease free control subjects within the required age-limit, we did not age-standardize the patients and controls. In addition, no significant difference was observed in the presentation of oxidative stress in relation to ethnic groups and sex among the healthy patients. However the results for control subjects have not been discussed extensively in the discussion section due to our primary interest of looking into the ethnicity variation of oxidant-antioxidant enzymes in elderly Type 2 DM patients with macrovascular complications.

Analytical methods

Venous blood samples were drawn in EDTA and plain tubes from fasting subjects attending the diabetic clinic. Samples were immediately subjected to subsequent procedures within an hour of sampling. The blood samples were centrifuged at 1,000 g for 10 minutes. The resulting plasma from EDTA tubes was used for lipid peroxidation analysis according to the method of Ratty & Das (1996) with malondialdehyde production used as an index for lipid peroxidation. Washed red blood cells were obtained from the remaining blood before proceeding to the subsequent antioxidant enzymatic assays. Superoxide dismutase (SOD; EC 1.15.1.1) activity was determined by the method of McCord & Fridovich (1969); the method employs xanthine and xanthine oxidase to generate superoxide radicals and involves the inhibition of nitroblue tetrazolium reduction to form blue formazan product at 540 nm. Catalase (CAT; EC 1.11.1.16) was determined according Beers & Sizers (1952) and is based on decomposition of hydrogen peroxide at 240nm. Glutathione peroxidase (GPX; EC 1.11.1.19) was determined by coupled enzyme procedure with glutathione reductase by the modified method of Paglia & Valentine (1967).

The blood collected in plain tubes was allowed to clot and centrifuged to obtain serum which was used for xanthine oxidase activity assay, based on the method of Singh *et al.* (1987). All enzymatic assays in blood samples were estimated spectrophotometrically according to known established method. All samples were analyzed within 3 hours of blood collection. Fasting plasma glucose was analyzed with a Beckman II glucose analyzer (Fullerton, CA); with intra- and inter-assay CVs of 2 % and 3 % respectively. HbA1c was measured by an affinity chromatography (IMX, Abbott Laboratorie, Abbott Park, IL; inter-assay CV 6.5%).

Statistical analysis

All data were analyzed using SPSS 10.5 for windows (SPSS, Chicago, Il). Data are expressed as mean ± SD. Statistical significance between controls and diabetic patients were analyzed by student t-test. Differences in means between ethnic groups were analyzed using Kruskal-Wallis or Wilcoxon rank test. Correlations between diabetic variables and ethnicity with lipid peroxidation were studied by Pearson's correlation coefficients test and differences were considered significant when $P < 0.05$.

Results

Table 1 illustrates the clinical characteristic and biochemical details of study subjects. The diabetic patients were closely matched for age, gender and duration of disease. Fasting blood glucose and HbA_{1c} was poor in the diabetes group with macrovascular complications compared to control subjects. Indian patients had poor glycaemic controls (as seen from fasting blood glucose and HbA1c levels) compared to controls subjects. In addition, MDA levels (an indicator of lipid peroxidation) were significantly higher in diabetic patients compared to control subjects. A positive correlation exists between MDA level with age, disease duration, FBG and HbA_{1c} (Table 2) in the three ethnic groups. There was a mild increase in xanthine oxidase activity (Table 1) in diabetic patients for all three ethnic groups compared to controls (mean = 0.68 U/L, SD =

0.42). No significant difference was observed among the three ethnic groups for xanthine oxidase activity. Indian patients showed greater susceptibility to macrovascular complications with altered antioxidant enzyme activities and increased MDA level compared to Malay and Chinese patients.

Discussion

Macrovascular disease leads to an increased prevalence of coronary artery, peripheral vascular disease and stroke (Colwell, 1991). Long-term vascular complications still represent the main cause of morbidity and mortality in diabetic patients (King & Rewers, 1993). Oxidative stress is considered to play an important role in the aetiology of diabetic complications and has been reported higher in patients with diabetes (Collier *et al.*, 1990; Baynes, 1991; Giugliano *et al.*, 1995). A high incidence of macrovascular complications in diabetes has been found in Asians from the Indian subcontinent both in their country of origin and in countries to which they have migrated (Tan *et al.*, 1999). The present study has found an alteration in antioxidant enzyme activities namely SOD, catalase and GPX in diabetic patients with a significant decrease in Indians compared to Malays and Chinese ethnic groups. Thus, it appears that hyperglycemia causes oxidative stress, which leads to consumption of antioxidant capacity (Ceriello, 1997). Alternatively, inactivation or alteration of antioxidant enzymes by glycosylation in poorly controlled diabetes mellitus may give rise to increased lipid peroxidation. Evidence of increased lipid peroxidation has also been observed in the present study. Plasma malondialdehyde was higher in patients with Type 2 DM, and, in particular, Indian diabetic patients had significantly higher level of plasma MDA compared to other ethnic groups.

The positive correlations that exist between MDA with glucose and glycated haemoglobin indicates that glucose may contribute to increase of oxidative stress in diabetic patients through nonenzymatic glycation and glucose auto-oxidative (Kennedy *et al.*, 1997). However, the possibility that insulin may contribute to the generation of oxidative stress by inducing intracellular production of free radicals should not be ruled out (Ceriello *et al.*, 1993). Ethnic differences in the secretion of insulin have been observed (Mohan *et al.*, 1996). Snehalatha *et al.* (1994), have reported that secretion of insulin, in the fasting and stimulated states, is higher in normoglycemic Indians living in India and abroad. In

Table 1. Clinical characteristic and biochemical details of study subjects

Characteristics	Malay	Chinese	Indian	Controls
Number	62	58	60	60
Gender (M/F)	30/32	30/28	30/30	34/26
HbA_{1c} (%)	9.5 ± 2.4	9.8 ± 2.7	12.1 ± 2.3**	5.38 ± 0.20
FBG (mmol/L)	12.5 ± 3.8	12.7 ± 3.9	14.4 ± 4.2**	4.8 ± 0.3
Age (years)	69.5 ± 10.3	70.4 ± 9.5	69.7 ± 11.8	46.8 ± 11.3
Disease duration (years)	10 - 40	10 - 40	10 – 40	-
MDA (µmol/L)	5.68 ± 0.64*	5.47 ± 0.51*	6.02 ± 0.68*, **	2.8 ± 0.35
SOD (U/g Hb)	1372.42 ± 94.32*	1403.72 ± 98.34*	1107.63 ± 95.76*, **	2540.15 ± 92.42
CAT (U/g Hb)	172.35 ± 19.14*	170.44 ± 18.72*	159.32 ± 19.05*, **	200.17 ± 17.68
GPX (U/g Hb)	16.23 ± 5.46*	16.79 ± 5.21*	14.42 ± 5.08*, **	34.25 ± 5.07
XO (U/L)	0.82 ± 0.54	0.86 ± 0.59	0.93 ± 0.51	0.68 ± 0.42

Values are expressed in mean ± SD; *P < 0.05 compared to control subjects; **P < 0.001 based on analysis of difference between ethnic groups.

Table 2. Correlation coefficients between lipid peroxidation and diabetes parameters

Parameters	Plasma malondialdehyde (µmol/L)		
	Indian	Chinese	Malay
Glucose	0.442*	0.358*	0.343*
HbA_{1c}	0.480*	0.370*	0.361*
Duration of disease	0.702**	0.674**	0.658**
Age	0.610**	0.581**	0.576**

* P < 0.05; ** P < 0.01 compared by Pearson correlation coefficients test.

addition, diabetic control was poor among Indian patients and this probably accounts for the higher incidence of macrovascular complication among Malaysian Indians. The present study also has detected strong association between age and duration of disease with lipid peroxidation, thus confirming the report of Griesmacher *et al.* (1995), Sundram *et al.* (1996), and Tessier *et al.* (1999) on accentuation of oxidative stress by the effects of aging and disease duration. It is possible to suggest that the mechanism for higher lipid peroxide levels in diabetes is multifactorial. The anticoagulant used during blood sampling, the type and strength of acid used in the pre-treatment procedure, and the duration of heating makes the interpretation of plasma MDA results difficult (Nielsen *et al.*, 1997). In this context, the measurement of isoprostanes, which are specific peroxidation product of polyunsaturated fatty acids, is now recognized as the most reliable index for the assessment of lipid peroxidation in humans and has been used to evaluate the clinical efficiency of antioxidant treatment in several diseases (Mezzeti *et al.*, 2000). However, the results obtained in our study are comparable to numerous reports on plasma MDA levels both in diabetic patients as well as healthy individuals (Griesmacher *et al.*, 1995; Sundram *et al.*, 1996; Kennedy *et al.*, 1997; Nielsen *et al.*, 1997 Tan *et al.*, 1999; Tessier *et al.*, 1999), thus, clearly indicating the validity of our approach in using known quantitative methods.

Measurement of xanthine oxidase activity in vivo is difficult due to its short half-life and low concentration in human sera (Bergel & Bray, 1986). In sera of healthy persons, xanthine oxidase activity is absent or only present at very low levels (Singh *et al.*, 1986). In the present study, xanthine oxidase activity was higher in diabetic patients compared to controls and no significant difference was found within the three ethnic groups. Furthermore the results obtained were within the reference interval for serum xanthine oxidase activity (0-1.20 U/L) as reported by Singh *et al.* (1986). Preliminary experiments carried out in our laboratory have shown that xanthine oxidase activity could be elevated in an induced hyperglycaemic system (unpublished report). There has not been a detailed study on the role of xanthine oxidase in the pathophysiology of diabetic complication. Nielson *et al.* (1996) and Nakazono *et al.* (1991) have highlighted the significance of xanthine oxidase in ischemic reperfusion and hypertension patients. Although there is no solid explanation, the increased activity of xanthine oxidase in Malaysian diabetic patients further supports the role of free radicals in diabetes. Our finding of elevation of serum xanthine oxidase could possibly provide an indirect validation of the assay.

The prominent features of oxidative stress among elderly Malaysian Indians compared to the other two major ethnic groups are consistent with studies reported by Sundram *et al.* (1986). Several factors could account for the rise including dietary changes, obesity, familial predisposition, racial characteristic, stressful personality, biological factors and cigarette smoking. Wide inter-individual variation may exist regarding antioxidant capacity, thus affecting individual susceptibility against deleterious oxidative reactions (Anderson *et al.*, 1997). The reason for the similar pattern of oxidative stress among Malay and Chinese patients is still unclear but is probably due to the presence of identical susceptible genes between them. Many studies have confirmed the existence of a genetic contribution to the determination of diabetic complication. Paraoxonase genes are good candidates for an increase vascular risk and different polymorphisms of this gene have been studied in Western diabetic macrovascular patients (Ruiz, 1997). Such studies have not been conducted in this part of the region. On the other hand, it is also possible that other variables such as differences in socioeconomic status, level of education, success in achieving glycaemic control, access to medical services and geographical isolation may be involved in the incidence of oxidative stress among the three ethnic groups. Although good metabolic control should be beneficial in reducing macrovascular complications in Type 2 DM (Coutinho *et al.*, 1999), this issue of oxidant-antioxidant enzymes and oxidative stress and their role in macrovascular diseases of Type 2 DM still needs to be elucidated in further prospective studies. Recognition of these factors and study of their interaction would be useful for physicians and researchers alike to overcome the morbidity and mortality from macrovascular disease in developing and new industrialized nations such as Malaysia.

Acknowledgement

We gratefully acknowledge the support of doctors, staff patients and volunteers at diabetic clinic, University Malaya Medical Centre. This investigation was funded by the Ministry of Science, Technology and Environment of Malaysia IRPA grant 06-02-03-0580, awarded to Dr Umah Rani Kuppusamy and collaborators. We also would like to express out gratitude to Dr Mahmood Ameen Abdullah and Mr Chai Jen Wai for their technical assistance.

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