ORIGINAL ARTICLE

Alteration of Lipid Peroxidation and Antioxidant Enzymes in Young Malaysian IDDM Patients

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Summary

The present study was designed to explore the relationship between lipid peroxidation and antioxidant enzymes in young Malaysian insulin dependant diabetes mellitus (IDDM) patients. Indicative parameters of lipid peroxidation, activities of antioxidant enzymes and diabetes parameters were evaluated in single blood samples from 30 young type 1 diabetic patients and 30 healthy control subjects. Antioxidant enzymes namely superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) were significantly decreased while plasma malondialdehyde (MDA), an indicator for lipid peroxidation was significantly increased in IDDM patients compared to control subjects. Positive correlations between HbA1c and MDA; fasting blood glucose (FBG) and MDA and negative correlations between HbA1c and SOD; MDA and SOD were observed in these patients. No significant correlation existed between HbA1c and fasting blood glucose, GPx or CAT in the diabetic patients. The strong correlations found between lipid peroxidation, antioxidant enzymes and diabetes parameters confirms the existence of oxidative stress in our IDDM patients.

Key Words: Insulin dependant diabetes mellitus, Oxidative stress, Antioxidant enzymes, Lipid peroxidation

Introduction

Diabetes is a progression of several events that occur over 10 to 15 years period[1]. There has been great progress in handling the short-term complications but not with the long-term complications. Type 1 diabetes mellitus usually begins in childhood with a lifelong tendency for hyperglycaemia and a high risk of developing serious long-term complications[1,2]. Emerging evidences suggest that oxidative stress plays an important role in the impairment of insulin action and insulin secretion[3]. Possible sources of oxidative stress in diabetes include decreased level of antioxidant enzyme activities and increased generation of reactive oxygen species (ROS), which leads to lipid peroxidation and glycation[3]. While the biochemical significance of oxidative stress has been understood for some time, the estimation of oxidative stress in vivo is quite difficult[4] with no standardization available. The measurement of antioxidant enzymes activities namely superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) and malondialdehyde (MDA), a parameter of lipid peroxidation have been acknowledged as tools for the assessment of oxidative damage in vivo[3,4]. Little is known about the interrelationship between these factors with the pathogenesis of diabetes complications in young Malaysian Type 1 diabetic patients. Such knowledge is of potential importance due to increased prevalence of diabetes in Malaysia with a high incidence of vascular, renal and visual complications. The current study was

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undertaken to assess the susceptibility of oxidative stress in Malaysian Type 1 diabetic patients. We also investigated the correlations between oxidative stress and diabetes parameters.

Materials and Methods

Patients

We studied about 30 IDDM patients attending our Diabetic clinic at University Malaya Medical Centre (UMMC), Kuala Lumpur. All patients were classified clinically as Type 1 according to the World Health Organization (WHO) study group report (1985) recommendations[5] with a mean age of 27.0±6.23 and duration of diabetes more than five years. The clinical records of the IDDM patients in this study were reviewed and five of them had clinically evident microvascular disease. The characteristics of study subjects are shown in Table I. Healthy volunteers (n=30) were recruited from staff and student populations of University Malaya after careful assessment and examinations. Since we failed to get enough disease free volunteers, we did not age standardize the patients and controls. Informed consent was obtained from all the participants of the study and the protocol was approved by the ethical committee of the medical centre.

Reagents

All reagents and chemicals were purchased from Sigma (St. Louis, Missouri; USA), Merck (Germany) and Fisher Scientific (UK) and were of the highest purity available.

Sample Preparation

Venous blood samples were drawn from fasting subjects attending the diabetic clinic. Sampling vials (5ml) containing fluoride oxalate as preservative was used for glucose measurement while EDTA coated vials (10ml) were used for HbA1c and MDA determinations. The remaining packed red blood cells in the EDTA tubes were then suspended in normal saline (0.9% Sodium Chloride) and centrifuged at 900g for three minutes. This washing procedure was repeated thrice. The washed red blood cells were then used for the antioxidant enzymes extraction and its subsequent assays. Preparation of washed red blood cells and biochemical analysis were done immediately after blood collection.

Clinical laboratory measurement

Fasting plasma blood glucose was analyzed with Beckman II glucose analyzer (Fullerton, California, USA); with intra and inter-assay CVs of 2% and 3% respectively. HbA1c was measured using microparticle enzyme immunoassay (MEIA); with interassay CVs of 6.5%.

Analytical Method

The enzyme assays and lipid peroxidation in blood samples were estimated spectrophotometrically (Shimadzu UV-1601) according to known established methods with slight modifications to suit our laboratory condition. Lipid peroxidation level was determined according to the method of Ratty and Das[6] based on thiobarbituric acid reaction in which malondialdehyde level (an index for lipid peroxidation) was measured at 532nm and was quantitated using extinction coefficient of $1.56 \times 10^5 m^{-1}cm^{-1}$. The intra- and interassay coefficients of variation for this method were 6.6 and 8.7% respectively. Superoxide dismutase (SOD; EC 1.15.1.1) activity was determined by the modified method of McCord and Fridovich[7]. This method employs xanthine and xanthine oxidase to generate superoxide radicals and involves inhibition of nitroblue tetrazolium reduction to form a blue formazan product, which absorbs at 540nm. Glutathione peroxidase (GPx; EC 1.11.1.19) activity was determined according to the method of Paglia and Valentine[8] by coupled enzyme procedure where glutathione reductase (EC 1.6.4.2) (GSSG-R) catalyses glutathione oxidation by H_2O_2. In the presence of glutathione reductase and NADPH, the oxidized glutathione is immediately converted to the reduced form with concomitant oxidation of NADPH to $NADP^+$ which can be measured at 340nm. Catalase (CAT; EC 1.11.16) activity was determined according to the method of Beers and Sizer[9] and it is based on the decomposition of hydrogen peroxide at 240nm.

Statistical analysis

All data were analyzed using SPSS 10.5 for Window (SPSS, Chicago, IL). Data are expressed as mean ± SD. Statistical significance between controls and diabetic patients were analyzed by analysis of variance (ANOVA). Correlation between variables were studied by Pearson's correlation coefficients and differences were considered significant when $P< 0.05$.

Results

Table I shows the clinical characteristics and biochemical data of IDDM patients and control subjects. The fasting blood glucose and glycated haemoglobin was significantly higher ($P<0.05$) in diabetes group compared to control subjects. The

antioxidant enzyme activities of SOD, GPx and CAT were significantly decreased while plasma MDA was significantly increased in IDDM patients compared to control subjects. Pearson's correlation analysis as shown in Table II, indicated that positive correlation exists between HbA1c and MDA (r =0.414, P<0.05); FBG and MDA (r = 0.432, P<0.05). Meanwhile a negative correlation exists between HbA1c and SOD (r = -0.374, P<0.05); SOD and MDA (r = -0.351, P<0.05) in IDDM patients. However, no correlation was found between fasting blood glucose and HbA1c, CAT and GPx. Ethnic variation in relation to the parameters measured were not assessed in this study due to the small sample size.

Table I: Clinical characteristics and biochemical details of study subjects

Characteristic	IDDM	Controls	P-value
N	30	30	NS
Sex (m/f)	17/13	19/11	NS
HbA1c (%)	9.5 ± 2.1	4.1 ± 0.3	P<0.001
FBG (mmol/L)	10.3 ± 4.7	4.3 ± 0.4	P<0.001
Age (years)	27.0 ± 6.23	27.3 ± 0.47	NS
MDA (μmol/L)	4.07 ± 0.26	2.7 ± 0.47	P<0.001
SOD (U/g Hb)	2154.41 ± 108.40	2680.77 ± 108.62	P<0.001
CAT (U/g Hb)	158.65 ± 18.83	175.29 ± 20.41	P<0.05
GPX (U/g Hb)	28.48 ± 5.92	34.55 ± 5.63	P<0.05

Values are expressed as mean ± SD.

The P-values refer to the difference from the control group. NS= not significant.

Table II: The correlation coefficients between antioxidant enzymes, lipid peroxidation and diabetes parameters

Parameters	Type 1 Patients
HbA1c / MDA	r=0.414, p<0.05
Glucose / MDA	r=0.432, p<0.01
HbA1c / SOD	r=-0.374, p<0.05
SOD / MDA	r=-0.351, p<0.05
HbA1c / GPx	r=-0.180
HbA1c / Catalase	r= -0.078
Glucose / GPx	r= -0.036
Glucose / Catalase	r= -0.040

Data are given as mean ± SD. P<0.05 compared by Pearson's correlation and linear regression.

Discussion

Many studies have shown that increased oxidative stress is present in diabetic subjects [3,10,11]. Consistent with this view, our data provides further evidence that there is presence of oxidative stress with an alteration in antioxidant enzyme activities and increased lipid peroxidation (MDA levels) in Type 1 diabetic patients. Similar results were also observed for Type 2 diabetic patients[12]. Furthermore, the values obtained for antioxidant enzyme activities and lipid peroxidation levels in this study were comparable to the results reported in Turkish[13] and French populations[14]. The significance of the positive relationship between HbA1c and FBG with lipid peroxidation is a matter for speculation. Although, high glucose concentration in vitro and hyperglycaemia in vivo are well known stimuli for generation of reactive oxygen species, the exact mechanism by which elevated blood glucose leads to lipid peroxidation is not known. Cerrielo et al[11] reported that despite hyperglycaemia, only a portion of the population of Type 1 diabetes will progress to diabetic nephropathy indicates an individual diversity in cellular response to high glucose concentration. The decrease in activities of antioxidant enzymatic system in diabetes is linked to progressive glycation of enzymatic proteins[10,14]. The negative correlation that was observed between SOD with HbA1c and MDA is an interesting finding. The decrease in SOD could possibly be due the inactivation of the enzyme active site by glycation[15]. In the absence of SOD, superoxide reacts with H_2O_2 to form the strongest biological oxidant known, the hydroxyl radical that could amplify the propagation of lipid peroxidation[7,15]. Our results enable us to speculate that the decreased SOD activity together with poor glycaemic control play a significant role in the cellular oxidative stress. The concentration of free radicals that exceeds the capacity of cellular antioxidant system results in increased lipid peroxidation products (MDA). It is intriguing that activities of SOD are significantly correlated with HbA1c while the other two-antioxidant enzymes GPx and catalase showed non-significance correlation with HbA1c and fasting blood glucose. It is possible to speculate that significant activation of the enzyme as a consequence of early oxidative stress and glycaemic control[16] might exert its effect at different level of enzymatic antioxidant system. Further studies are needed to verify this speculation. Alternatively our data also suggest that increased oxidative stress in IDDM patients is associated with the diabetes itself. Conceivably, the increased oxidation further supports the role of oxidative stress as an early stage in disease pathology[17] whereby it pre-dates the complication and is not simply a consequence of the complications.

Many indirect methods have been proposed and are generally used to assess in vivo oxidative stress[15,18]. We sought to take advantage of known established quantitation methods that are easier, economical and sensitive. The future of antioxidant therapy in diabetes seems promising with various studies being carried out in several laboratories around the world. Recently, researchers at the National Jewish Medical Centre and University of Colorado Health Centre have developed a synthetic antioxidant that can protect insulin producing beta cells from lethal oxygen radicals generated in diabetes[19]. Moreover it has been shown that peroxiredoxins, a recently discovered family of antioxidant protein is more inducible by oxidative and nitrostative stress in rat pancreatic beta cell. The beta cells are known to express low concentrations of SOD, GPX and CAT[20] and are not able to protect the cells against nitrostative stress. These findings, we hope would open up a very promising new line of research.

Conclusion

This study shows the existence of oxidative stress in the development of diabetic complication in Malaysian population. Further studies are needed to clarify the relationship between lipid peroxidation and antioxidative function and their pathophysiological significance in diabetes. Designing an effective preventive measure would require a good understanding of the mechanism and link between genetic susceptibility with non-genetic factors in Type 1 diabetes.

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