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New Coronary Risk Factors: Is There a Difference between Diabetic Patients with Microalbuminuria Compared to Those without Microalbuminuria?

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Summary

Diabetes mellitus is an important risk factor for the development of coronary artery disease. The presence of microalbuminuria, which indicates renal involvement in diabetic patients, influences the progression of coronary artery disease. New coronary risk factors such as C-reactive protein (CRP), Lipoprotein a [Lp (a)] and fibrinogen are increasingly being recognized as important cardiovascular prognostic factors. These new coronary risk factors could account for the worse cardiovascular prognosis in diabetic patients with microalbuminuria. Our cross sectional study was to compare the prevalence of elevated CRP and the levels of Lp (a) and fibrinogen between diabetic patients with microalbuminuria and those without microalbuminuria. Diabetic patients with overt coronary artery disease were excluded from the study. A total of 108 patients were recruited of which 57 patients had microalbuminuria and 51 were without microalbuminuria. There was no difference in the number of patients with elevated CRP between these two groups. There were also no significant differences in the mean values of Lp (a) and fibrinogen between diabetic patients with and without microalbuminuria. The inflammatory marker CRP and coagulopathy markers i.e. Lp (a) and fibrinogen seem not to be perturbed in diabetic patients with microalbuminuria.

Key Words: New coronary risk factors, Diabetes mellitus, Microalbuminuria

Introduction

Both insulin dependent diabetes mellitus (IDDM) and non insulin dependent diabetes mellitus (NIDDM) are conventional risk factors for the development of coronary artery disease (CAD).

Atherosclerosis occurs earlier in diabetic patients compared to the general population[1]. Although the exact cause of the accelerated atherosclerosis is not known, a few postulations have been made. Hypertension and obesity are common in patients with diabetes, and these may contribute

to the atherosclerosis process. The nonenzymatic glycation of lipoproteins may be an important factor too[2]. The procoagulant environment that exists in diabetes mellitus is another possible explanation for the accelerated atherosclerosis. Increased platelet adhesiveness and aggregability has been demonstrated[3].

Microalbuminuria is the earliest clinical sign of diabetic nephropathy. A number of studies have suggested that microalbuminuria is associated with increased cardiovascular risk and early cardiovascular mortality in type 2 diabetic patients[4-6]. The apparent association of microalbuminuria with coronary artery disease could be related to the presence of adverse risk profile in these patients. Patients with microalbuminuria were found to have higher blood pressure and total cholesterol levels as compared to those without microalbuminuria. However the study by Mattock et al[4] showed that the presence of microalbuminuria in type 2 diabetes mellitus was an independent risk factor for coronary artery disease.

New coronary risk factors that have strong predictive power for the occurrence of coronary artery disease have been identified in the general population. Lipoprotein (a) [Lp (a)], which is a variant of low-density lipoprotein, has been identified as one of the new risk factors for CAD[7, 8]. Lp (a) is found to be increased in IDDM patients especially those with microalbuminuria or poor glycaemic control[9]. Its level is however not raised in NIDDM patients. Data are scarce relating Lp (a) to coronary artery disease among diabetics.

Another new coronary risk factor that has been of increasing interest to cardiologists is fibrinogen. Fibrinogen, which is a thrombogenic factor, has been shown to be an independent CAD risk factor[10]. An increase of each standard deviation of fibrinogen was associated with 30% and 40% increase in the coronary events in men and women respectively in the Framingham Heart Study Population[11]. Elevated levels of fibrinogen have been associated with diabetes and greater atherosclerotic risk in population studies[12].

C-reactive protein (CRP), which is an acute phase reactant, is another new CAD risk marker. In the Framingham Heart Study population, elevated CRP level was associated with an increased risk for the development of coronary events[11]. Tracy et al.[13] also found an association between elevated baseline CRP and the incidence of coronary events in a prospective, nested case-control study.

We undertook this study to determine whether there were any differences in the levels of these new coronary risk factors in diabetic patients with and without microalbuminuria. Our research hypothesis was that diabetic patients with microalbuminuria have increased atherogenetic process, an inflammatory process that may be detected by CRP, and also abnormal coagulation status as measured by Lp (a) and fibrinogen.

Materials and Methods

Our cross-sectional study recruited diabetic patients who were attending the diabetes and endocrinology clinic in Hospital UKM from July 1998 to January 1999. Male and female diabetic patients (IDDM and non IDDM) aged between 16 and 80 years old were included in the study. The criteria for the diagnosis of diabetes mellitus was fasting venous glucose level of more than 6.7 mmol/L or random venous glucose level of more than 10.0 mmol/L. The exclusion criteria included patients with hypertension (systolic and diastolic blood pressures of more than 140 and 90 mm Hg respectively), the presence of clinical (as determined by the Rose questionnaire) and electrocardiographic evidence of ischaemic heart disease, renal impairment (serum creatinine above 133 mmol/L), treatment with angiotensin converting enzyme (ACE) inhibitor or

antiarrhythmic drugs, recent acute illness of less than two weeks duration and history of alcohol intake of more than 2 units per day.

Patients who fulfilled the criteria had had their blood taken for the measurement of CRP, fibrinogen and Lp (a) after an eight-hour fast. Quantitative measurement of albumin in the urine was carried out by an enzyme-linked immunosorbent assay using kits (Exocell Inc., Philadelphia). The intra-assay coefficient of variations (CV) at 6.1 and 51.0 µg/ml were 6.4 and 7.4% respectively, with the corresponding inter-assay CVs were 14.2 and 12.8% respectively. Urine creatinine was determined colorimetrically using reagents supplied by Roche Products Ltd UK. Microalbuminuria was defined as albumin: creatinine ratio of 2.5-25 mg/mmol for male and 3.5-25 mg/mmol for female, and/or albumin excretion rate of 20-200 µg/min or 30-300 mg/24 hour.

HBA1c was measured by HPLC using Variant Analyzer, Bio-Rad Laboratories where normal reference was 4-6%. C-reactive protein was measured by the immunoturbidimetric method using a Cobas Integra analyser. The working range was between 0.5-16 mg/dl with a sensitivity of 0.5 mg/dl. The intra-assay CVs at 5.3 mg/dl and 11.4 mg/dl were 2.0% and 2.4% respectively, with the corresponding inter-assay CVs at 1.7% and 1.4% respectively. C-reactive protein was considered elevated if 0.5 mg/dl or more. Lp (a) and fibrinogen were measured using the immunoturbidimetric method performed on Cobas Mira analyser (Roche Diagnostic). The working range of Lp (a) was between 0.1-1.5 g/l with a sensitivity of 0.05 g/l. For the measurement of Lp (a), the intra-assay CVs at 0.39 g/l and 0.8 g/l were 3.9% and 2.3% respectively, with the corresponding inter-assay CVs of 5.4% and 6.2% respectively. Lp (a) concentration was considered raised if above 0.3 g/l. Fibrinogen concentration was considered raised if above 3.0 g/l.

Statistical Analysis
For data with a Gaussian distribution, the comparison between means was determined using the Student's t-test. Data with non-Gaussian distribution, such as Lp (a) was logarithmically transformed before Student's t-test was performed. Values were expressed as mean (± SD) or geometric mean (± SD) where appropriate. Chi square or Fisher exact test, where necessary was used to compare discrete variables between the groups. A p value of less than 0.05 was considered statistically significant. All statistical analyses were performed with the SPSS Statistical Software (version 10, SPSS Inc.).

Results

A total of 108 consecutive diabetic patients (46 males, 62 females) who fulfilled the inclusion criteria were recruited in the study. The patients' age ranged from 17 years to 77 years old with a mean of 45.7 years. A total of 18 patients (16.7%) were insulin dependent diabetes mellitus (IDDM) patients while 90 patients (83.3%) were non-insulin dependent diabetes mellitus patients (NIDDM). The glycosylated hemoglobin (HbA1c) level of the patients ranged from a minimum of 4.9% to a maximum of 15.1% with a mean level of 8.8%.

There were 57 patients (52.8%) with microalbuminuria compared to 51 patients (47.2%) without microalbuminuria. There was no significant difference in the mean age of patients with microalbuminuria compared to those without microalbuminuria (46.9 ± 12.8 years vs 44.4 ± 13.3 years, p = NS). There was also no significant difference in the mean HbA1c level between patients with microalbuminuria and those without microalbuminuria (8.8 ± 2.4%, p = NS). Comparisons between patients with microalbuminuria are shown in Table I.
Seven out of 57 patients (12.3%) with microalbuminuria had raised CRP levels

compared to 2 out of 51 patients (3.9%) without microalbuminuria who had elevated CRP level (Table II). Crosstabulation using Fisher's Exact test did not show any association between elevated CRP level and the presence of microalbuminuria (p = 0.167).

There was no significant difference in the mean value of fibrinogen in patients with microalbuminuria compared to those without microalbuminuria (1.63 $\pm$ 0.12 g/l vs 1.68 (0.13 g/l, p = NS) (Table III). A total of 4 out of 51 patients (7.8%) with microalbuminuria had raised fibrinogen levels (above 3.0g/l) compared to 3 out of 48 patients without microalbunuminuria

(6.3%) who had raised fibrinogen. There was no association between raised fibrinogen level and the presence of microalbuminuria (p = NS).

There was also no significant difference in the log mean value of Lp (a) between patients with and without microalbuminuria (-0.81 $\pm$ 0.41 vs -0.79 $\pm$ 0.42, p = NS). The mean value of Lp (a) of the two groups of patients is shown in Table III. A total of 16 out of 56 patients with microalbuminuria (28.6%) had raised Lp (a) (> 0.3g/l) compared to 17 out of 51 patients without microalbuminuria (33.3%). There was no association between raised Lp (a) and the presence of microalbuminuria.

Table I: Baseline characteristics of patients with microalbuminuria and those without microalbuminuria

	Microalbuminuria (n = 57)	Non Microalbuminuria (n = 51)	p-value
Mean age (years)	46.9 $\pm$ 12.8	44.4 $\pm$13.3	NS
Male	22	22	NS
Female	33	29	NS
IDDM	6	12	NS
NIDDM	51	39	NS
HbA1c (%)	8.8 $\pm$ 2.1	8.6 $\pm$ 2.4	NS

Table II: Comparison of the number of patients with raised CRP

	Microalbuminuria	Non microalbuminuria	p-value
Number of patients with CRP > 0.5 mg/dl	7/57 (12.3%)	2/51 (3.9%)	NS

NS indicates not significant

Table III: Mean value of fibrinogen and Lp(a) in patients with and without microalbuminuria

	Microalbuminuria	Non microalbuminuria	p-value
Fibrinogen (g/l)	1.63 $\pm$ 0.12	1.68 $\pm$ 0.13	NS
Lipoprotein (a)(g/l)	0.24 $\pm$ 0.03	0.27 $\pm$ 0.04	NS
Log Lp (a)	-0.81 $\pm$ 0.41	-0.79 $\pm$ 0.42	NS

* Mean values are expressed in mean $\pm$ SD
NS indicates not significant.

Discussion

We did not find any differences in the levels of the new coronary risk factors between diabetic patients with and without microalbuminuria. Both the mean values of CRP and fibrinogen, which were acute phase proteins, were not elevated in diabetic patients with microalbuminuria compared to those without microalbuminuria. The number of patients with elevated CRP and fibrinogen also did not differ significantly between these two groups of patients. This is in contrast to the study by Schmitz et al.[14] who showed that the fibrinogen level was higher in diabetic patients without microalbuminuria. Lp (a), which had been identified as an independent risk factor for the development of premature coronary artery disease in men, was also not significantly different between diabetic patients with microalbuminuria and in those without microalbuminuria. Lp (a) has been found to be increased in IDDM patients especially among those with microalbuminuria or poor glycaemic control in other studies[9].

Our study had excluded the diabetics with both clinical and ECG evidence of ischaemic heart disease. We had wanted to examine whether the diabetic patients with microalbuminuria and without overt clinical evidence of coronary artery disease had increased levels of these new coronary factors. The elevated levels of these new coronary markers, if present, could possibly explain for the increased risk of coronary artery disease in the diabetic patients with microalbuminuria. A few reasons could possibly explain for the lack of difference between these two groups of diabetics in our study. As none of our patients had any overt clinical features of coronary artery disease, the finding of elevated level of acute phase reactant such as CRP would

be highly unlikely. Moreover, the presence of subclinical ischaemic heart disease, if any, would still denote that the atherosclerotic plaque was stable as none of these patients had any prior clinical events. In these circumstances, it is not likely to find elevated levels of acute phase reactants such as CRP and fibrinogen. It would have been better if we had managed to analyze the levels of CRP below 0.5 mg/dl as continuous variables rather than labeling the CRP as elevated if it was above this value. We were hampered in this aspect by our laboratory machine, which could not quantify the values of CRP below 0.5 mg/dl. The small number of patients in our study could also possibly account for the non-significant differences in fibrinogen and Lp (a) levels between the diabetic patients with and without microalbuminuria. The classification of diabetic patients into IDDM and NIDDM patients might be useful to determine the prevalence of elevated Lp (a). Previous published studies had shown that Lp (a) to be increased in IDDM patient especially those with microalbuminuria but not in NIDDM patients[9]. A larger diabetic study population is needed if this were to be done.

Despite these limitations, our study suggest that microalbuminuria might not be the determinant of elevated levels of new coronary risk factors in diabetics without clinical evidence of coronary artery disease. We further postulate that the stability of an atherosclerotic plaque and the type of diabetes mellitus might influence the elevation of these new coronary risk factors.

Acknowledgments

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