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GLYCEMIC CONTROL MODIFIES THE ASSOCIATION BETWEEN MICROALBUMINURIA AND C-REACTIVE PROTEIN IN TYPE 2 DIABETES MELLITUS

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ABSTRACT

Microalbuminuria and C-reactive protein reflect closely related components of the same disease process. The present study attempts to evaluate whether any association exists between C-reactive protein and microalbuminuria in Type 2 Diabetes Mellitus patients with poor and adequate glycemic control. It was observed that in diabetics with poor glycemic control, microalbuminuria showed a significant positive correlation with C-reactive protein and the prevalence of microalbuminuria was significantly more at elevated C-reactive protein levels. These parameters were not significant in subjects with adequately controlled disease. Further, there was a significant increase in levels of microalbuminuria in patients with poor glycemic control when compared to well-controlled diabetics at comparative levels of C-reactive protein. This study supports the hypothesis that endothelial dysfunction and inflammatory activity are involved in the pathogenesis of microalbuminuria and underscores the importance of glycemic control in the progression of inflammation in diabetes.

KEY WORDS

Microalbuminuria, C-reactive protein, Glycemic control, Diabetes Mellitus

INTRODUCTION

Microalbuminuria (MAU) is an established marker of diabetic nephropathy(1,2). It begins insidiously and may precede the diagnosis of Type 2 Diabetes Mellitus (DM 2), occurring with the insulin resistance syndrome and its components, including obesity and hypertension(3). It is estimated that a duration of greater than 6 years of DM 2 may have existed before the diagnosis(4).MAU refers to the excretion of albumin in urine at a rate that exceeds normal limits but is less than the detection level of traditional dipstick methods(5). Results are expressed as mg/24 hours urine specimen, µg/minute in a timed urine specimen or as µg/mg Creatinine in a random spot urine test(5). The appearance of albumin in urine is thought to be the consequence of generalized endothelial damage along the vascular tree including the glomerulus(6).

In addition to its established role as an early indicator of diabetic nephropathy, recent findings suggest that it may be an independent marker of cardiovascular risk(7,8). Indicators of cardiovascular risk such as markers of inflammation, have been shown to be associated with MAU in population of patients with or without DM(9). C-reactive protein (CRP) is one of the most sensitive markers of sub-clinical inflammation and is thought to represent a state of chronic low-grade inflammation of the arterial wall(10). CRP has been found to consistently predict cardiovascular prognosis(11) and has been reported to be a potent risk factor for coronary heart disease(12,13). Measurement of this acute-phase reactant is being routinely used to detect and monitor inflammatory changes and interventions that reduce CRP levels have been found to decrease the occurrence of coronary events(14,15).

Because CRP and MAU reflect closely related components of the same disease process, a strong relationship between these two variables may be anticipated. But information available about their mutual association and its modification with glycemic control in diabetics is scanty. With rising world-wide prevalence of DM 2, markers of future disease risk and complications would allow earlier prevention strategies to be tested. Before MAU and CRP can be used in a preventive strategy, more needs to be known about the mechanisms of association between elevated excretion of urinary albumin,

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glucose intolerance, other inflammatory parameters and their relative contributions to cardiovascular and renal complications. The present study attempts to evaluate whether any association exists between CRP and MAU in well-controlled and poorly controlled Type 2 Diabetes Mellitus patients. The findings may find usefulness in preventing or hindering the development of long-term complications of DM.

MATERIALS AND METHODS

The subjects of the present study were selected from patients attending the outpatient department of R.L.Jalappa Hospital, Kolar, Karnataka during the period July 2005 to December 2005. Subjects diagnosed as suffering from Type 2 Diabetes Mellitus according to the report of the expert committee for diagnosis and classification of Diabetes Mellitus (16) was included in the study. DM 2 was defined as diabetes treated by diet alone or by diet combined with oral hypoglycemic agents or as treatment with insulin in a case of diabetes onset after the age of 40 years (17). Inclusion criteria contained the following parameters: age of onset > 40 years of age, serum Creatinine < 1.5 mg/dl, serum triglyceride < 400 mg/dl, absence of proteinuria with the dipstick test, negative urine culture and no evidence of hypertension, cardiovascular and renal disease or any other chronic disease requiring therapeutic intervention. All the participants who attended the outpatient clinic gave written informed consent.

The subjects were divided into two groups according to glycemic control as Group 1 (Type 2 Diabetes Mellitus subjects with poor glycemic control) and Group 2 (Type 2 Diabetes Mellitus subjects with adequate glycemic control), based on their levels of plasma glucose, both in fasting condition and two hours after ingestion of 75g glucose, and glycated hemoglobin as per the following criteria:

Group 1 (Type 2 Diabetes Mellitus subjects with poor glycemic control) :
Fasting plasma glucose (FPG) > 126 mg/dl,
2 hour post-prandial glucose (PPG) > 200 mg/dl after a 75g glucose load,
Glycated hemoglobin (GHb) of > 10%.

Group 2 (Type 2 Diabetes Mellitus subjects with adequate glycemic control) :
Fasting plasma glucose (FPG) < 110mg/dl,
2 hour post-prandial glucose (PPG) < 140 mg/dl after a 75g glucose load,
Glycated hemoglobin (GHb) of < 8 %.

All patients with impaired glucose homeostasis (FPG from110 to 126 and 2 hour PPG from 140 to 200) were excluded from the study.

Each group was further subdivided into subgroups A, B and C based on CRP levels. Thus, the groups 1 and 2 (based on Glycemic control) were further divided into 3 subgroups A, B and C (based on CRP level) with 15 subjects in each of these six subgroups as follows:

1A: Poorly controlled Diabetics having a CRP level between 0 – 3 mg/L,
1B: Poorly controlled Diabetics having a CRP level between 3 – 6 mg/L,
1C: Poorly controlled Diabetics having a CRP level between 6 – 9 mg/L,
2A: Well-controlled Diabetics having a CRP level between 0 – 3 mg/L,
2B: Well-controlled Diabetics having a CRP level between 3 – 6 mg/L,
2C: Well-controlled Diabetics having a CRP level between 6 – 9 mg/L.

In order to provide for equal representation and randomization in each subgroup, the first 15 subjects conforming to criteria of each group were included in that group. In this way, out of a total of 163 subjects primarily screened, 90 subjects falling into 6 subgroups (with 15 in each subgroup) were finally included in the study.

Levels of plasma glucose, glycated hemoglobin, C-reactive protein and microalbuminuria in study groups 1 and 2 were evaluated by standardized assay procedures. Other relevant laboratory parameters were also measured. The results were tabulated and subjected to statistical analysis.

Urinary albumin excretion was measured by Microalbuminuria – turbilatex quantitative turbidimetric test for the measurement of MAU in human urine (Spinreact S.A., Spain). MAU was defined as an excretion rate of albumin between 30-300 mg/24 hours (20-200 µg/min) which is above normal values but still below values seen in conventional proteinuria. MAU was determined on 12-14 hours overnight urine collected during 2 consecutive days.

CRP levels were measured with a semi quantitative latex test (Span Diagnostics, India) with a sensitivity of 3 mg/l. The normal reference value of CRP was taken as < 6 mg/l.

Plasma glucose estimation was carried out after 12-14 hours

of overnight fasting for FPG value and 2 hours after a 75g glucose load for PPG value. Plasma glucose was measured by standard Glucose oxidase – peroxidase reaction system (Zydus Pathline, Cadila). Glycated hemoglobin was assayed in blood by standard method employing cation exchange resin (Pointe Scientific). Normal reference range was taken as 6.0% to 8.3%.

RESULTS

The parameters of Fasting plasma glucose (FPG), 2 hour post-prandial glucose (PPG) and Glycated hemoglobin (GHb) in both groups were obtained as shown in Table 1.

Table 1 : Fasting plasma glucose (FPG), 2 hour post-prandial glucose (PPG) and Glycated hemoglobin (GHb) in groups 1 and 2.

Group 1 (Type 2 Diabetes Mellitus subjects with poor glycemic control)		
	(Mean ± SD)	Range
Fasting plasma glucose	166 ± 28 mg/dl	129 – 197 mg/dl
2 hour post-prandial glucose	281 ± 67 mg/dl	205 – 357 mg/dl
Glycated hemoglobin	11.1% ± 0.8%	10.1% - 12.2%
Group 2 (Type 2 Diabetes Mellitus subjects with adequate glycemic control)		
	(Mean ± SD)	Range
Fasting plasma glucose	79 ± 21 mg/dl	51 – 107 mg/dl
2 hour post-prandial glucose	102 ± 26 mg/dl	68 – 136 mg/dl
Glycated hemoglobin	6.4 % ± 0.9%	5.3% - 7.8%

Table 2 depicts detailed observations in each of the 6 study cohorts. As shown in this table, subjects with poor glycemic control had elevated values of MAU at all comparative levels of CRP. Further, it was shown that the level of MAU increased considerably with increasing level of CRP.

The relationship between MAU levels and CRP in diabetics with poor glycemic control (Group 1) is given in Table 3. A significant positive correlation (pearson's correlation coefficient 0.667, $p < 0.01$) between these two variables was found to exist in these patients. However, in diabetics with adequate glycemic control (Group 2) these variables failed to show any significant correlation (pearson's correlation coefficient 0.342, $p > 0.05$) as denoted in Table 4.

Table 5 shows the comparative values of MAU between subjects of groups 1 and 2 at comparable levels of CRP (ie. Between 1A and 2A, 1B and 2B, 1C and 2C). It was found that there is a significant increase ($p < 0.05$) in MAU in subjects with poor glycemic control in respect to the well-controlled diabetics at all comparable levels of CRP.

Table 2 : Microalbuminuria (MAU) and C-reactive protein (CRP) status in poorly controlled and adequately controlled Type 2 Diabetes Mellitus.

GROUP 1				
Sub Group	CRP (mg/L)	MAU (mg/24hours) Mean ± SD	Range	% positives
1A	0 – 3	78.30 ± 45.72	12 – 172	50
1B	>3 – 6	150.0 ± 59.30	63 – 220	100
1C	>6 – 9	208.9 ± 62.94	88 – 291	100
GROUP 2				
Sub Group	CRP MAU (mg/L)	(mg/24hours) Mean ± SD	Range	% positives*
2A	0 – 3	27.60 ± 10.86	7 – 45	40
2B	>3 – 6	28.30 ± 13.89	8 – 56	50
2C	>6 – 9	39.90 ± 17.41	13 – 73	50

* % positive refers to % of subjects excreting albumin in urine at a rate of 30 – 300 mg/24hrs.

Table 3 : Relationship between MAU and CRP levels in Type 2 DM patients with poor glycemic control.

Group	CRP (mg/L)	MAU (mg/24hrs) Mean ± SD	Pearson's Correlation	Significance
1A	0 – 3	78.30 ± 45.72		
1B	>3 – 6	150.0 ± 59.30	0.667	P < 0.01*
1C	>6 – 9	208.9 ± 62.94		

*Positive correlation is significant at 0.01 level.

Table 4 : Relationship between MAU and CRP levels in Type 2 DM patients with adequate glycemic control.

Group	CRP (mg/L)	MAU (mg/24hrs) Mean ± SD	Pearson's Correlation	Significance
2A	0 – 3	27.60 ± 10.86		
2B	>3 – 6	28.30 ± 13.89	0.342	P = 0.065*
2C	>6 – 9	39.90 ± 17.41		

*No significant correlation.

The relative percentages of prevalence of significant MAU at different levels of CRP are given in Table 6. The percentage prevalence of significant MAU was defined as the percentage of subjects in the study group excreting albumin in urine at a rate between 30 – 300 mg / 24hours. From the data, it was inferred that compared to group 2 subjects, the patients of group 1 were more likely to have significant MAU in presence of abnormally elevated CRP. In group 2, the increase is only

Table 5 : Comparative study of MAU between patients of groups 1 and 2 at similar levels of CRP: Results of independent t test.

CRP (mg/L)	Group	MAU (mg/24hrs) Mean ± SD	t value	p value
0 – 3	1A	78.30 ± 45.72	2.407	P < 0.05*
	2A	27.60 ± 10.86		
>3 – 6	1B	150.0 ± 59.30	6.318	P < 0.01*
	2B	28.30 ± 13.89		
>6 – 9	1C	208.9 ± 62.94	8.183	P < 0.01*
	2C	39.90 ± 17.41		

*All differences significant at 0.05 levels.

Table 6 : Percentage prevalence of significant MAU* at normal and abnormal levels of CRP in groups 1and 2: results of student's t test for testing difference in proportions

Group	CRP (mg/L)	% prevalence of significant MAU*	% increase in prevalence
1A and 1B	0 – 6	50	100
1C	6 – 9	100	
2A and 2B	0 – 6	40	25
2C	6 - 9	50	

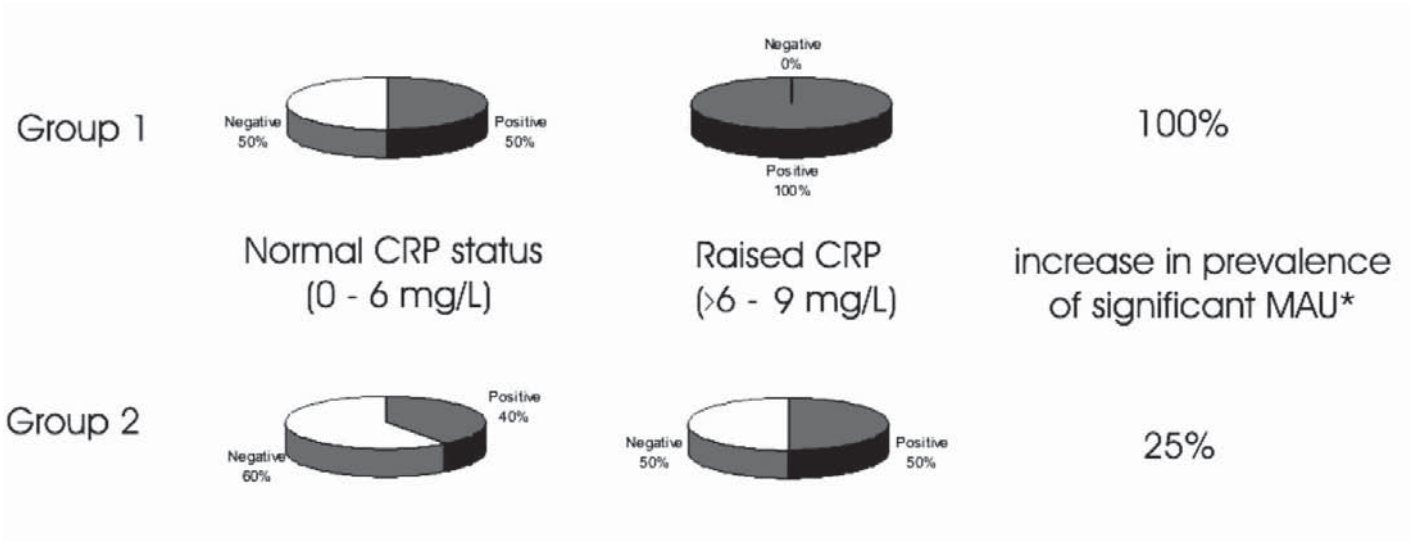
*Significant MAU refers to excretion of albumin in urine at a rate of 30 – 300 mg/24hrs.; **Difference in proportion of % increase in MAU positive cases with abnormal CRP levels between groups 1 and 2 is significant at 0.05.

25% (from 40% to 50%) between patients with normal and abnormal CRP levels whereas in group 1, this prevalence increased by 100% (from 50% to 100%). This difference was found to be significant by on the student's 't' test for testing difference in proportions (t = 3.42, p < 0.01). The corresponding composite graphical representation is given in the form of pie-charts in figure 1.

DISSCUSSION

The present study was conducted to evaluate whether any association exists between CRP and MAU status in type 2 DM patients and if such association is modified significantly by glycemic control. It was observed that in diabetics with poor glycemic control, MAU showed a significant positive correlation with CRP level (pearson's correlation coefficient 0.667, p < 0.01). On the other hand, among subjects with adequately controlled disease, the trend in association between CRP and MAU was not significant (pearson's correlation coefficient 0.342, p > 0.05). A similar association between CRP and MAU has been reported in a large cross-sectional study by Festa et al (18). This study population contained patients with and without DM 2. They reported a positive association between MAU and elevated CRP which was similar across gender and ethnic groups. Also, it was found that mean levels of CRP was significantly more in microalbuminuric subjects than normoalbuminurics. In another study, Stehouer et al (19) prospectively followed the markers of chronic inflammation and endothelial dysfunction in DM 2 patients. They found a strong interrelation between markers of both chronic

Figure 1 : Pie-charts showing prevalence of significant MAU* at normal and raised CRP levels in groups 1 and 2



*Significant MAU refers to excretion of albumin in urine at a rate of 30 – 300 mg/24hrs.

inflammation and endothelial dysfunction and MAU. This observation has been substantiated by Navarro et al (20).

Because MAU and CRP are shown to be closely associated in poorly controlled DM, the cause of this relationship has been hypothesized. Stuveling et al (21) reported that CRP was associated with MAU and decreased renal filtration as measured by creatinine clearance. These data raise the point that inflammatory processes may adversely affect renal function and hence MAU. Festa et al (18) have suggested that the association may arise due to independent development of inflammatory changes and MAU, direct or indirect effect of cytokine on the glomerulus or the presence of both from a pre-existing condition. Other workers (20) have supported the hypothesis that in addition to metabolic and hemodynamic factors, inflammation plays a key role in the pathogenesis of diabetic nephropathy. MAU has been shown to be consistently associated with endothelial low-grade inflammation (22, 23). Ross (10) has proposed that dysfunction of vascular endothelium and chronic low-grade inflammation are key features in the pathophysiology of atherothrombosis and MAU.

Stehouer et al (19) disagrees that this association is causal. They found no plausible mechanism directly linking atherothrombotic disease to quantitatively trivial albumin loss characteristic of MAU. Instead they proposed that MAU is a marker of pathophysiological process that causes both increased renal albumin loss and atherothrombosis. Thus they have suggested that MAU reflects severity of atherosclerosis (26). These findings suggest that chronic inflammation could emerge as a potential mediator between MAU and macrovascular disease.

The modifying effect of glycemic control on the association between CRP and MAU as illustrated in the present study is also supported by work of other researchers. Guerrero et al (27) conducted a study to determine the relationship between CRP levels and components of metabolic syndrome including MAU in normal glucose tolerant (NGT), impaired glucose tolerant (IGT) and Type 2 diabetics. They found that with NGT patients, CRP was not associated with MAU. IGT subjects and diabetics showed a significant association between CRP and MAU. From these findings, they concluded that there is a significant relationship between CRP and MAU in diabetics and IGT. Subjects with NGT failed to show any significant association.

Stehouwer et al (19) has shown that both endothelial dysfunction and inflammation are involved in the pathogenesis of MAU and poor glycemic control was associated with increase in markers of endothelial dysfunction and inflammatory activity. They observed that HbA_{1c} was consistently positively associated with longitudinal development of markers of inflammatory activity and endothelial dysfunction. It has been postulated that the association may reflect combined biological effects of hyperglycemia, Amadori products and advanced glycated end products (28,29). Also, acute hyperglycemia has been shown to increase IL-6, TNF-α and IL-18 (30). These findings suggest that prevention of hyperglycemia and other anti-inflammatory treatments may be beneficial in addressing the early progressive inflammatory response associated with diabetes and vascular disease.

The results of the present study showed a significant increase in MAU of patients with poor glycemic control when compared with well-controlled diabetics at comparative status of CRP. Similar findings has been noted in the study by Navarro JF et al (20) in which multiple regression analysis showed that urinary albumin excretion was significantly associated with duration of diabetes and glycated hemoglobin level. Regression of MAU with proper glycemic control has also been reported. Perkins et al (31) has observed that regression of MAU was associated with a glycated hemoglobin level of less than 8%. Regression was defined as a 50% decrease in albumin excretion occurring over one 2-year study period to the next and occurred in 58% of these patients. These findings support the observations of the present study.

It may be concluded from the results of this study that:

1. There is a significant positive correlation between the level of MAU and CRP status in poorly controlled Type 2 diabetics.
2. This correlation is absent in diabetics with adequate glycemic control.
3. MAU is significantly more in patients with poor glycemic control than in well-controlled diabetics at similar levels of CRP.
4. Compared to patients with well-controlled disease, the subjects with poor glycemic control were twice more likely to have MAU in presence of elevated CRP.

It may be summarized that poor glycemic control increases the risk of complications of DM 2 in part through causing endothelial dysfunction, increased inflammatory activity and MAU. These phenomenons are interrelated and their association with development of complications is mutually interdependent. This data provides a basis for investigating

the effects of specific intervention to decrease inflammatory activity and improve endothelial function in DM 2 and support the hypothesis that endothelial dysfunction and inflammatory activity are involved in the pathogenesis of MAU as well as underscores the importance of proper glycemic control in arresting the progression of inflammation.

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