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EVALUATION OF ASSOCIATION AMONGST SERUM MAGNESIUM, HS-CRP AND MICROALBUMINURIA IN TYPE 2 DIABETES MELLITUS

Biochemistry

KEYWORDS: Magnesium, hsCRP, Microalbuminuria, Endothelial injury, Diabetes Mellitus

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ABSTRACT

High sensitive C-reactive protein (hs-CRP) is an established marker of low grade systemic inflammation. Nephropathy in type 2 diabetes mellitus (T2DM) develops due to chronic low grade inflammation and vascular endothelial dysfunction. Microalbuminuria (MAU) is thought be a result of generalized damage of the endothelium in T2DM. Low magnesium has a direct role in promoting endothelial dysfunction by generating a pro-inflammatory, prothrombotic and proatherogenic environment. Hypomagnesaemia has been implicated in adversely affecting diabetic complications. Because serum Magnesium, CRP and MAU reflect closely related component of the same disease process, a strong relationship between these variables may be anticipated. We selected 50 patients of Type-2 diabetes mellitus and 50 normal healthy individuals to evaluate this association. The statistical analysis depicted a highly significant positive ($r=0.506$; $p<0.001$) correlation between hs-CRP and microalbuminuria, a highly-significant ($p< 0.001$) negative correlation ($r=-0.57$) between microalbuminuria and serum magnesium and a non-significant ($p> 0.05$) negative correlation ($r=-0.20$) between serum magnesium and hs-CRP in type 2 diabetic patients. This data supports the hypothesis that inflammatory activities and hypomagnesemia are involved in the pathogenesis of MAU while hypomagnesemia and hs-CRP may be involved independently in the pathogenesis of diabetes and its complications.

INTRODUCTION: Type 2 diabetes mellitus is now recognized as an inflammatory condition associated with insulin resistance and abnormal endothelial vascular reactivity. [1] Independent of the triggering agent and of the initial events, any process linked to chronic inflammation which decrease insulin action and insulin resistance will lead to worsening of inflammation in a vicious cycle. One of the most sensitive acute-phase reactants in humans is hs-CRP. [2][3]

Microalbuminuria is an established marker of diabetic nephropathy. Microalbuminuria is thought to be the consequence of generalized endothelial damage along the vascular tree. [4] Microalbuminuria is defined as an excretion of albumin in the urine, amount ranging from 30 to 300 mg/day or 20 to 200 mg/L. [5] It begins insidiously and may precede the diagnosis of type 2 DM [4] It is at this stage that one can hope to reverse diabetic nephropathy or prevent its progression.

There is accumulating evidence that the altered metabolism of some micronutrients in diabetes mellitus might have a specific role in the pathogenesis and complications of the disease. Magnesium deficiency is the most evident disturbance of metal metabolism in insulin-dependent diabetes mellitus. Mg depletion negatively affects the glucose homeostasis and insulin sensitivity in diabetic patients [6] as well as the complications such as retinopathy, thrombosis and hypertension. [7] Clinically, hypomagnesaemia is defined as a serum Mg concentration less than or equal 1.6 mg/dl or >2 SD below the mean of the general population. [8] [9] Preventing hypomagnesaemia may therefore be beneficial in the management of the diabetics. [10] Low Magnesium levels may promote endothelial cell dysfunction and thrombogenesis via increased platelet aggregation and vascular calcification.[11]

The development of formula based calculation of estimated glomerular filtration rate (eGFR) has offered a very practical and easy approach for converting serum creatinine value into GFR result; taking into consideration patient's age, sex, ethnicity and weight (depending on equation type). In 2000 Levey *et al* subsequently published a 4-variables (4-v MDRD) equation that does not require albumin and urea with no impact on accuracy. [12] Both Increased urinary albumin excretion (albuminuria) and reduced GFR are risk factors for progressive kidney failure and cardiovascular disease. [13]

Because serum magnesium, hs-CRP and MAU seem to be closely related component of the same disease process, a strong relationship between these variables may be anticipated.

MATERIALS AND METHODS: The present study was a case control prospective study undertaken in the Department of Biochemistry in collaboration with Department of Medicine, Sri Guru Ram Das Institute of Medical Sciences and Research, Amritsar. A total of 100 subjects willing to participate in the study with informed consent were included in the study. 50 patients of poorly controlled Type 2 Non Insulin Dependent Diabetes Mellitus (NIDDM) between 40-65 yrs of age, of either sex whose HbA1c was >7% and 50 healthy, age and sex matched controls from the same population but without any disease and without family history of DM.

Exclusion criteria: Patients suffering from type-1 DM, patients with acute complications of DM like Diabetic ketoacidosis, history of acute infections, other ailments like gross congestive heart failure, tuberculosis, gout, rheumatoid arthritis and skeletal muscle injury, serum creatinine > 1.5mg/dl, renal failure and those giving positive dip stick test for proteinuria were not included in the study.

The patients and controls were screened for fasting blood sugar (FBS), lipid profile, serum Magnesium, serum hs-CRP and microalbuminuria and the values were compared with that of normal healthy subjects. Serum Magnesium was estimated by Calmagite method. [14] Hs-CRP was estimated by Quantia –CRP (M A Mendall et al 1996) [15], a turbidimetric immunoassay. Microalbumin in urine was estimated by Nycocard Reader (Diabetes Care 1997) [16] GFR was estimated from serum creatinine using the Modification of Diet in Renal Disease (MDRD) equation.[17] FBS and lipid profile were estimated by kit methods. Serum creatinine was estimated by jaffes kinetic method (Watchtel et al 1995) [18] and its calibrator has been standardized to ID-MS.

Result: There was no significant effect of age ($p>0.05$) and sex distribution ($p > 0.05$) in the study. Table 1 shows that FBS, HbA1c, total cholesterol, triglyceride, hs-CRP and MAU levels were significantly high while Mg and HDL-C were significantly low in cases as compared to the controls. Whereas no significant difference was found in the values of creatinine and eGFR in cases when compared to controls.

Table 1 : Comparison of various parameters estimated in patients and controls

	Cases (Mean±SD)	Controls (Mean±SD)	p value
Fasting blood sugar (mg/dl)	194.38 ± 53.60	100.30 ± 12.46	< 0.001*

HbA1c (%)	8.758 ± 1.83	5.148 ± 0.51	< 0.001*
Total cholesterol (mg/dl)	223.20 ± 45.41	174.46 ± 33.90	< 0.001*
Triglycerides(mg/dl)	224.70 ± 76.77	161.14 ± 32.42	< 0.001*
HDL-C(mg/dl)	40.48 ± 8.18	55.00 ± 12.04	< 0.001*
Magnesium (mEq/L)	1.09 ± 0.29	2.09 ± 0.29	<0.001*
hs-CRP (mg/dl)	1.29 ± 1.79	0.57 ± 0.09	< 0.005**
Albumin in urine (mg/L)	35.36 ± 15.36	18.28 ± 1.47	<0.001*
Serum creatinine (mg/dl)	1.09 ± 0.257	1.05 ± 0.318	0.502***
eGFR (ml/mim/1.73 m^2)	68.28 ± 24.83	68.54 ± 31.87	0.201***

*P<.001 =highly significant
**p<0.05-significant
***p>0.05- non significant

Table 2 shows that FBS and HbA1c were significantly correlated with Mg, hs-CRP and MAU in type 2 diabetics. Total cholesterol, triglycerides and HDL-C had no significant correlation with hs-CRP but a significant correlation with Mg and MAU in type 2 diabetic patients.

Table 2: Correlation of serum Magnesium, hs-CRP and MAU in type 2 Diabetes Mellitus with all other parameters

	Magnesium (mEq/L))	hs –CRP (mg/dl)	MAU (mg/L)			
	R	P	R	P	R	P
Fasting blood sugar (mg/dl)	-0.541	<0.001*	0.404	0.004**	0.47	<0.001*
HbA1c (%)	-0.320	<0.001*	0.432	0.002**	0.60	<0.001*
Total cholesterol (mg/dl)	-1.89	0.01**	0.017	0.906***	0.36	0.01**
Triglycerides(mg/dl)	- 0.011	<0.001*	0.151	0.294***	0.48	<0.001*
HDL-C(mg/dl)	0.176	0.033**	-0.051	0.724***	-0.30	0.033**

*P<.001 =highly significant
**p<0.05-significant
***p>0.05- non significant

Table 3: Correlation of serum hs-CRP with microalbumin in urine and serum Magnesium in patients of type 2 diabetes mellitus (cases)

Parameter	Mean ± SD	R	P
Microalbumin in urine (mg/L)	35.36 ± 15.36	0.506	0.001*
Magnesium (mEq/L)	1.09 ± 0.29	-0.20	0.163 ***

*P<.001 =highly significant
***p>0.05- non significant

Table 3 shows a highly significant positive correlation of hs-CRP with microalbuminuria (r=0.506; p <0.001) while a non significant negative correlation with Magnesium (r=-0.20, p > 0.05)

Table 4: Correlation between serum magnesium and microalbumin in urine in patients of type 2 diabetes mellitus (cases)

Parameter	Mean ± SD	R	P
Magnesium (mEq/L)	1.09 ± 0.29	-0.47	0.001*
Microalbumin in urine (mg/L)	35.36 ± 15.36		

*p< 0.001 - highly significant

Discussion: In present study significantly high levels of hs-CRP (p=0.004) were observed in T2DM than controls (Table 1) and supported by other studies. [19] [20] The biological mechanisms through which hs-CRP increases risk of type 2 diabetes is not well understood. Hs-CRP may have indirect influence on insulin resistance and insulin secretion through altered innate immune response. [21] No significant correlation between hs-CRP and lipids profile parameter may suggest that hs-CRP act as an independent marker for cardiovascular risk factor (Table2)

Albumin excretion in urine in type 2 diabetic patients was significantly high than in controls (Table 1). These findings were supported by various other studies. [22] [23] This can be interpreted as an early sign of nephropathic changes in the diabetic patients. It could possibly be due to the degradation of the glomerular basement membrane, particularly its surface layer, the glycocalyx. [22] Increasing evidence suggests that the progression of insulin resistance to type 2 diabetes parallels the progression of endothelial dysfunction to atherosclerosis. [24] A significant correlation of microalbuminuria with lipid parameters suggests that MAU may be related to insulin resistance in the prediction of cardiovascular events.

In our study, significantly low levels of magnesium were found in patients as compared to controls (p<0.001) supported by other studies. [25] [26] [27] Various clinical studies demonstrated that diabetics with hypomagnesemia showed reduced pancreatic β-cell activity. Insulin receptor autophosphorylation is dependent on intracellular Mg^2+ concentrations, making Mg^2+ a direct factor in the development of insulin resistance. Conversely, insulin is an important regulator of Mg^2+ homeostasis. In the kidney, insulin activates the renal Mg^2+ channel melastatin type 6 that determines the final urinary Mg^2+ excretion. Consequently, patients with T2DM and hypomagnesemia enter a vicious circle in which hypomagnesemia causes insulin resistance and insulin resistance reduces serum Mg^2+ concentrations. [28] [29] [30] Patients with hypomagnesemia show a more rapid disease progression and have an increased risk for diabetes complications. [31] Preventing hypomagnesemia may therefore be considered in the management of the disease. [32] No significant correlation between magnesium and lipids profile parameter may suggest that magnesium act as an independent marker for cardiovascular risk factor.

The statistical analysis in our study showed that there existed a highly significant positive (r=0.506; p <0.001) correlation between hs-CRP and microalbuminuria in type 2 diabetic patients. (Table 3) According to Fen-qin Chen et al [19] when compared with the normal albuminuria group, levels of the inflammatory cytokines (hs-CRP, TNF-α, uMCP-1, and SAA) in T2DM patients were significantly higher in the microalbuminuria and macroalbuminuria group,p < 0.01. In another study by Doung II et al [33], both MAU-D (MAU in diabetes) and MAU-H (MAU in hypertensives) groups, hs-CRP levels were positively correlated with MAU level (P < 0.05). In their study by Q. Liu et al [34], the hs-CRP concentrations in diabetic nephropathy (DN) patients were significantly higher than that in controls of healthy people and diabetes mellitus (DM) patients without nephropathy. The appearance of albumin in urine is thought to be the consequence of generalized endothelial damage along the vascular tree including the glomerulus. In addition, only one study has explored the relation of CRP or endothelial dysfunction with albuminuria in elderly diabetics and revealed that microalbuminuria is associated with markers of endothelial dysfunction in elderly normotensive type 2 diabetic patients. [35]

In our study, no significant difference in the values of eGFR in cases and control was found (Table 1). This may be because the increase Microalbuminuria precedes a fall in glomerular filtration rate in patients developing diabetic chronic kidney disease (CKD). [36] [37]

In our study, we found a highly significant negative correlation between serum magnesium and microalbuminuria (p<0.001) (Table 3). This was supported by others. [38] Low Mg levels may promote endothelial cell dysfunction and thrombogenesis via increased platelet aggregation and vascular calcifications. [8] Low Mg levels also

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may lead to the induction of proinflammatory and profibrogenic response reduction of protective enzymes against oxidative stress, [39] induction or augmentation of vasoconstriction and hypertension and stimulation of aldosterone among others. Moreover, because Mg is crucial in DNA synthesis and repair, it is possible that Mg deficiency may interfere with normal cell growth and regulation of apoptosis. [40] Because microalbuminuria is related to endothelial dysfunction and increased oxidative stress, it is not surprising that diabetic atherosclerosis parallels diabetic glomerulosclerosis and is a very powerful risk factor for coronary heart disease and stroke in diabetic persons.[24]

Very few studies have been conducted on the analysis of the correlation between serum Magnesium and hs-CRP in the type 2 diabetic patients. In our study, on statistical analysis there was non-significant (p> 0.05) negative correlation (r=-0.20) between serum magnesium and hs-CRP. This data supports the hypothesis that hypomagnesemia and hs-CRP may be involved independently in the pathogenesis of diabetes and its complications which might require further evaluation. One drawback in our study can be small sample size. A study by G Romero et al (2011) [41] showed that severe hypomagnesemia (Odds Ratio (OR): 8.1 and Confidence Interval (CI) 95%: 3.6-19.4 and OR: 3.7; CI 95%: 1.1-12.1) was strongly associated with elevated hs-CRP in patients of metabolic syndrome.

Conclusion: In type 2 diabetic patients, microalbuminuria is significantly associated with elevated hs-CRP levels. This data provides a basis for investigating the effects of specific intervention to decrease inflammatory activity and improve endothelial function in type 2 diabetes. This supports the hypothesis that endothelial dysfunction and inflammatory activity are involved in the pathogenesis of MAU. This study may also highlight the importance of proper glycemic control in arresting the progression of inflammation. Magnesium might be linked to various micro and macro vascular complications. A better understanding of magnesium metabolism and efforts to minimize hypomagnesemia in routine management of diabetes are warranted. No significant association between serum magnesium and hs-CRP supports the hypothesis that hypomagnesemia and hs-CRP may be involved independently in the pathogenesis of diabetes and its complications which might require further evaluation.

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