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EVALUATION OF ASSOCIATION BETWEEN SERUM HS-CRP AND MICROALBUMINURIA IN TYPE 2 DIABETES MELLITUS

Biochemistry

KEYWORDS: hs-CRP, Microalbuminuria, Nephropathy, Dibetes 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. Because hs-CRP and MAU seems to be closely related component of the same disease process, a strong relationship between these variables may be anticipated. The statistical analysis depicted a highly significant positive (r=0.506; p <0.001) correlation between hs-CRP and microalbuminuria. This data supports the hypothesis that inflammatory activities are involved in the pathogenesis of MAU.

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.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.[6] Both Increased urinary albumin excretion (albuminuria) and reduced GFR are risk factors for progressive kidney failure and cardiovascular disease.[7]

Because hs-CRP and MAU seems 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 hs-CRP and microalbuminuria and the values were compared with that of normal healthy subjects. Hs-CRP was estimated by Quantia –CRP (M A Mendall et al 1996) [8], a turbidimetric immunoassay. Microalbumin in urine was estimated by Nycocard Reader (Diabetes Care 1997) [9] NycoCard U-Albumin is a solid phase, sandwich-format, immunometric assay. GFR was estimated from serum creatinine using the Modification of Diet in Renal Disease (MDRD) equation.[10] FBS was estimated by GOD-POD Method (Trinder 1969)[11]. HbA1C by Nycocard Reader (Jeppson 2002) [12] Total Serum Cholesterol was be estimated by CHOD-PAP Method (Allain C.C. et al 1974) [13] Serum Triglyceride was be estimated by GPO-Trinder Method. (McGowan MW et al 1983) [14] Serum High Density Cholesterol (HDL-C) was estimated by Phosphotungstic Acid Method (Gordon T. Et al 1977) [15] Serum creatinine was estimated jaffes kinetic method (Watchtel et al 1995) [16] 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, HDL-C, hs-CRP and MAU levels were significantly high in cases when compared to 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*
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 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 MAU in type 2 diabetic patients.

Table 2: Correlation of hs-CRP and MMAU in type 2 Diabetes Mellitus with all other parameters

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

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

Table 3: Correlation between microalbumin in urine and serum hs-CRP 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*
hs-CRP (mg/dl)	1.29 ± 1.79		

*P<.001 =highly significant

Table 3 shows a highly significant positive (r=0.506; p <0.001) correlation between hs-CRP and microalbuminuria

Discussion: In present study significantly high levels of hs-CRP (p=0.004) were observed in T2DM than controls (Table 1). A similar observation was noted by Safiullah et al [17], Fen-qin Chen et al [18],

Zhen Wang et al [19] 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.[20]

A highly significant positive correlation was found between hs-CRP and FBS as well as HbA1c (Table 2) supported by the other studies. [17]

Observational studies have also suggested an independent role of hs-CRP in development of insulin resistance and diabetes, but it is unclear whether the association is a causal one or the consequence of inefficacy of the adipose tissue and other confounding factors.[22]

In our study, there existed non significant correlation between hs-CRP and total cholesterol, triglyceride and HDL (Table 2) which was supported by Chen Chung Fu et al [21] and D A Muttur et al [22] No significant correlation between hs-CRP and lipids profile parameter may suggest that hs-CRP act as an independent marker for cardiovascular risk factor.

In present study, 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] In our study we found a highly significant positive correlation between FBS, HbA1c and MAU. DA Muttur et al [22] and Choudhary N et al [25] showed a strong positive correlation (p <0.001) between FBS and MAU and FBS and HbA1c respectively. Increasing evidence suggests that the progression of insulin resistance to type 2 diabetes parallels the progression of endothelial dysfunction to atherosclerosis. [24] In our study (Table 2) we found a significant positive correlation of microalbuminuria with total cholesterol and triglycerides while a significant negative correlation with HDL. It can be argued that MAU may be related to insulin resistance in the prediction of cardiovascular events.

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) This was supported by the studies Choudhary N et al [25] and M J Mojahedi et al [26] which also reported a significant positive correlation between hs-CRP and microalbuminuria in type 2 diabetic patients. According to Fen-qin Chen et al [18] when compared with the normal albuminuria group, levels of the inflammatory cytokines (hs-CRP, TNF- , uMCP-1, and SAA) in T2DM patients were significantly higher in the microalbuminuria and macroalbuminuria group, < 0.01. In another study by Doung II et al [27], 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 [28], 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. [21]

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).[29] [30]

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.

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