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"Association of C-reactive protein with renal functions and microalbuminuria in type 2 diabetes mellitus"

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Introduction: *Estimation of microalbumin levels in urine has been the gold standard for monitoring the diabetic nephropathy progression and is also predictive of high HbA1C levels.MAU is a preliminary manifestation of diabetic nephropathy which initiates as a result of microvascular changes.*
Objective: *To assess the renal functions (Urea, Creatinine and Uric Acid) and to evaluate the levels of CRP association of CRP with MAU,Urea, Creatinine and Uric Acid in type 2 DM.*
Result: *mean ACR levels Uric acid, CRP levels was significantly higher in poor glycemic control patients significant correlation coefficient were observed between HbA1C & CRP level was 0.539 (P=0.000), between HbA1C & MAU was (r=0.237,(P=0.017), CRP and ACR was (r=0.325,P=0.000).*
Keywords:*CRP levels, ACR,type 2 diabetes mellitus*

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I. Introduction

Diabetes is a major cause of morbidity and mortality throughout the world especially more alarming in developing countries.Diabetes is among the leading causes of kidney failure (Global status report on non-communicable diseases 2010. Geneva, World Health Organization, 2011)and screening for early signs of diabetes – related to kidney disease (nephropathy) is a cost saving intervention and feasible for developing countries. Microvascular complications including nephropathy, retinopathy and neuropathy are initiated by chronic hyperglycemia.

In diabetic patients, glycemic control i.e. maintaining the normal blood sugar levels, plays a very significant role in averting the risk of developing both acute & chronic complications.

Several studies have suggested a strong correlation between level of hyperglycemia and the progression of microvascular complications in diabetic patients (Battisti WP et. al., 2003). According to the study by Steouwer C et. al., 2002 both endothelial dysfunctions and inflammation are involved in the pathogenesis of microalbuminuria (MAU) and poor glycemic control was associated with increase in markers of endothelial dysfunction and inflammatory activity. They observed that HbA1C was consistently positively associated with longitudinal development of markers of inflammatory activity and endothelial dysfunction (Stehouwer C et. al., 2002).

C-reactive protein (CRP) is a glycoprotein is one of the most sensitive markers of subclinical inflammation and is thought to represent a state of chronic low grade inflammation of the arterial wall. CRP, named for its capacity to precipitate the somatic C-polysaccharide of streptococcus pneumonia, was the first acute-phase protein to be described and is an exquisitely sensitive systemic marker of inflammation and tissue damage (Pepys MB et. al., 2003).

Estimation of microalbumin levels in urine has been the gold standard for monitoring the diabetic nephropathy progression and is also predictive of high HbA1C levels (Nosadini R et. al., 2003). MAU is a preliminary manifestation of diabetic nephropathy which initiates as a result of microvascular changes.

The present study was therefore planned to assess the "Association of C-reactive protein with renal functions and microalbuminuria in type 2 diabetes mellitus with the following objectives:To assess the renal functions (Urea, Creatinine and Uric Acid) and to evaluate the levels of CRP association of CRP with MAU,Urea, Creatinine and Uric Acid in type 2 DM.

II. Materials and Methods

The study was conducted in Department of Biochemistry in association with Department of General Medicine & Endocrinology of Mahatma Gandhi Medical College & Hospital, Jaipur. A study protocol was approved by the Institutional Ethics CommitteeTotal 100 patients Diagnosed cases of type 2 DM.Age Criteria <60 years either gender were taken into the study.Pregnant and lactating women,patients with alcoholic habit, drug dependence, urinary tract infection, patients who had any major surgery within 4 weeks of screening were excluded.

Method

Blood samples after overnight fasting were collected by standard aseptic techniques.
Fasting & Post-prandial blood samples were collected and subjected to estimation of blood sugar, HbA1C, CRP, RFT (Urea, Creatinine and Uric Acid),Microalbuminuria and ACR(Using VITROS 4600 - Dry Chemistry Analyzer)
Urea, Creatinine and Uric acid. Spot urine samples were collected for estimation Albumin Creatinine Ratio.

Specimen Handling and Storage

- Handle and store specimens in stoppered containers to avoid contamination and evaporation.
- Mix samples by gentle inversion and bring to room temperature, 18–28 °C (64–82 °F), prior to analysis.

Based on the results obtained, the patients enrolled for the study were group according to S.CRP level as –

- CRP ≤ 10.0 mg/L
- CRP > 10.0 mg/L

The subjects enrolled for the study were further grouped based on the glycemic control. According to the American Diabetes Association, Patients with HbA1C levels ≤ 8.0% are considered as good glycemic control whereas those with HbA1C >8.0% are said to have a poor glycemic control.
The groups formed were:

- HbA1C ≤ 8.0% (good control) n=48
- HbA1C > 8.0% (poor control) n=52

Calculation of Albumin-Creatinine Ratio (ACR):

Albumin-Creatinine Ratio (ACR) (µg/mg):

$$\frac{Albumi\ ninurinespotspecimen \quad (mg/L)}{Creatinineinurinespotspecimen \quad (\frac{mg}{dl})} \text{x} 100$$

(American Diabetes Association. Diabetic nephropathy, Diabetes Care 2003)

STATISTICAL ANALYSIS

Different variables were presented as mean ± SD in the two groups and compared by applying students 't' test. Correlation between different variables were evaluated by applying Pearson's correlations.A P-value ≤ 0.05 was considered as statistically significant.

III. Results

Out of 100 patients male : female distribution was 57 : 43.The patients were distributed into two groups based on CRP levels. 31 patients had the level of CRP ≤ 10.0mg/L while 69 patients had CRP > 10.0mg/L.In the subgroups 18 male and 13 female had CRP ≤ 10.0mg/L while 38 male and 31 female had CRP > 10.0mg/L.The mean age in the groups based on CRP levels was comparable & found to be non-significant.

The fasting and post-prandial blood sugar levels were significantly varying in two groups with higher levels in the groups with CRP > 10.0mg/L.The HbA1C levels were also significantly higher (P=0.000).The mean ACR levels were significantly higher in the positive CRP group based on CRP levels (P=0.006).The mean levels of urea, creatinine and uric acid were significantly higher in positive CRP groups and elevation of uric acid was highly significant (P=0.000) as compared to creatinine and urea.

Further the patients were grouped according to HbA1C levels. out of the 100 patients enrolled, 48 patients had HbA1C ≤ 8.0% and 52 patients had HbA1C > 8.0%.In the group HbA1C ≤ 8.0%, out of 48 patients, 52% were male & 48% were female.In the poor glycemic control group HbA1C > 8.0%, 60% were male and 40% patients were female. The mean age of the subjects were comparable in two groups (P=NS).Fasting and PP blood sugar levels were significantly higher in the groups based on HbA1C levels (P<0.001S).The mean HbA1C levels in the groups based on poor & good glycemic control was significantly

higher (P<0.001S).The CRP levels were significantly higher in the poor glycemic control group than good glycemic control group (P<0.001S).The mean levels of ACR is high in poor glycemic control group than good glycemic control groups. Thus it is also highly significant (P<0.001S).

Distribution of urea and creatinine were significant in poor glycemic control group than good glycemic control group.Uric acid was significantly higher in poor glycemic control patients.Out of 100 patients 38 patients had ACR ≤ 30.0 while 62 patients had ACR > 30.0. Of the total patients enrolled 56% male and 44% female patients has ACR >30.0.On applying Pearson's correlation, the significant correlation coefficient were observed between HbA1C & CRP level was 0.539 (P=0.000), between HbA1C & MAU was (r=0.237,(P=0.017),CRP and ACR was (r=0.325,P=0.000).

IV. Discussion

In fact, the mean HbA1C level in the negative CRP group is <8.0 which is considered as good glycemic control In a study by **Mojahedi M J et. al., 2009**, blood glucose levels were higher in the positive CRP group though the variations with that of negative CRP group were not statistically significant.

It was observed these three urea, creatinine & uric acid parameters were significantly higher in the positive CRP group. Elevation of uric acid was highly significant as compared to creatinine and urea.

Since CRP is a marker of inflammation &hyperuricemia is a predictor of endothelial dysfunction, both the above parameters have gained recognition as independent risk markers of atherosclerosis also well as cardiovascular complications (**Brownlee M et. al., 2003; VermaS et. al., 2002; Venugopal SK et. al., 2002**).

Previous study by **Tsioufis C et. al., 2006** has reported that elevated CRP level increases the risk of diabetes upto 2.7 times.

A risk in CRP ≤10.0mg/L along with uric acid levels may be indicative of patients at high risk of developing various complications (**Ishihara M et. al., 1989).**

Comparing the ACR in patients with positive and negative CRP levels, it was observed that ACR levels were significantly higher in association with elevated CRP levels.

Previous studies have reported a correlation between CRP levels and microalbumin in diabetic patients (**Brownlee M et. al., 2003). Mangiliet. al., 1998** suggested that low grade inflammation indicated by increased levels of hs-CRP may induce microalbuminuria& hence increase the risk of cardiovascular disease (**Mangili et. al., 1998).**

Persistent microalbuminuria (MAU) further is a strong predictor of diabetic nephropathy which if untreated can progress to kidney failure (**Venugopal SK et. al., 2002; Perkins BA et. al., 2003).**

Though within normal limits, significantly higher S. creatinine levels were reported in positive CRP group in the current study. This finding is suggestive that raised CRP levels are indicative of not only inflammatory changes but also influence the renal function in diabetic patients.

Student's 't' test was used to compare between the two groups. Out of the 100 patients enrolled, 48 patients had HbA1C ≤8.0% and 52 patients had HbA1C >8.0%.

The sugar levels were significantly varying in the 2 groups with higher levels in the group with HbA1C ≤ 8.0%.

No statistical significance was observedin HbA1C levels. & hence the subjects were comparable in the two groups.CRP levels were significantly higher in the poor glycemic control group than good glycemic control group (P<0.001S).

The mean level of ACR is significantly high in poor glycemic control patient than good glycemic control group (P=0.000).It was found that there is a significant increase in MAU in subjects with poor glycemic control group.

uric acid is significantly higher in the poor glycemic control patient while the difference of blood urea and S. creatinine is non-significant on the basis of formed glycemic control groups.

In various studies, it has been reported that there is a correlation between serum CRP levels and microalbuminuria in diabetic patients and even in the general population (**Brownlee M et. al., 2003).** These observations suggest that low grade inflammation, reflected by high serum hs-CRP levels, may play a role in the induction of microalbuminuria, which can be considered as a risk factor of cardiovascular disease (**Mangili R et. al., 1998; Mojahidi MJ et. al., 2009)**

In the study by **Festa A et. al., 2000**, the study population included patients with and without T2DM. They reported a positive association between MAU and elevated CRP which was similar across gender and ethnic group.

In our study it was found that mean levels of CRP was significantly more in microalbuminuric subjects than normoalbuminurics.

Stehouer C et. al., 2002 prospectively followed the markers of chronic inflammation & endothelial dysfunction in type 2 DM patients. They found a strong interrelation between markers of both chronic inflammation and endothelial dysfunction and MAU.

MAU & CRP are shown to be closely associated in poorly controlled DM, the cause of this relationship has been hypothesized. **Stuveling E et. al.,** 2003, 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.

In the present study there was a significantly increase of MAU in patients with poor glycemic control, when compared with well-controlled diabetes. Similar findings were reported by **Blessing O, Meera KS et. al., 2011,** in population based studies **(Meera KS et. al., 2011).**

Many complications arise due to uncontrolled or poorly controlled diabetes mellitus amongst which the most destructive is diabetic nephropathy **(Battisti WP et. al., 2003).** Several works have been done on the significance of MAU in the early diagnosis of diabetic nephropathy with correlation to other parameters like albumin to creatinine ratio in urine (**Vijay V et. al., 1994).**

Previous studies have also reported a significant positive correlation between CRP level and ACR (**Gonzalez L et. al., 1997; Ishihara M et. al., 1989).**

Also **Mojahedi M J et. al., 2009**, revealed in their study that MAU is positively correlated to elevated hs-CRP suggesting the measurement of serum hs-CRP as a screening method in future studies, to help in diagnosing early stages of diabetic neuropathy sooner and easier.

The National Kidney Foundation, 2007 recommends that patients with diabetes should be screened annually for diabetes kidney disease for diagnosis of T2DM. Screening should include measurements of urinary ACR in a spot urine sample and measurement of serum creatinine and estimation of GFR (National Kidney Foundation). KDOQI clinical practice guidelines and clinical practice recommendations for diabetes and chronic kidney disease (**Am J Kidney Dis 2007**). According to the latest guidelines, the **ADA 2008** recommends to perform annual test to assess urine albumin excretion in all type 2 Diabetic patients, starting at diagnosis.

In the past decades, it has become widely accepted that inflammation plays a key role in the pathogenesis of cardiovascular disease (CVD) **(Ridker PM et. al., 2000).**

Patients with Type 2 DM have markedly increased atherosclerotic risk. Recent observations in apparently healthy individuals have focused attention on inflammatory mechanism that may be relevant in patients with diabetes as well (**Dandona P et. al., 2003).**

A study in Tunisian population showed that elevated CRP levels was associated with an increased risk of type 2 DM, even after adjusting for age, gender, body mass index (BMI), smoking and alcohol consumption.

In the general Japanese population elevated CRP concentration was observed to be a significant predictor of diabetes, independently of obesity and insulin resistance **(Doi Y et. al., 2005).**

In the study of **Anwarullahet. al., 2014,** a statistically significant correlation was found between the prevalence of MAU and HbA1C level. This study explicitly indicated that poor diabetic control is the leading cause of diabetic nephropathy as evident by elevated MAU**.**The study therefore recommends that estimation of CRP may be useful in screening of diabetic patients and also nondiabetic patients with impaired glucose tolerance. An early diagnosis of elevated CRP levels may be helpful in timely patient management.

Further research to explore the levels of other inflammatory markers like hs-CRP, IL-6 etc. and their correlation with HbA1C and ACR is also proposed. The effect of other factors like obesity, BMI, lifestyle, smoking and alcohol consumption on the concentration of the above markers may also be interesting to study further.

Table 1: Comparative statistics among CRP ≤ 10.0 and >10.0

Group	CRP ≤ 10.0	CRP > 10.0	P-value
	n = 31	n = 69	
Male : Female	18:31(58.06% vs 41.93%)	38:31(55.07% vs 44.92%)	
Age (yrs.)	52.65±14.44	42.04±33.35	NS
BSF (mg/dl)	147.20±42.12	195.14±91.77	0.007S
BSPP (mg/dl)	186.00±97.26	300.67±65.77	<0.001S
HbA1C (%)	7.07±1.87	9.25±2.32	<0.001S
CRP (mg/dl)	6.72±2.24	42.04±33.35	<0.001S
ACR (mg/L)	27.06±16.02	53.82±51.48	0.006S
Urea (mg/dl)	29.06±18.09	39.21±24.23	0.04S
Creatinine (mg/dl)	0.74±0.21	1.09±0.71	0.009S
Uric acid (mg/dl)	4.21±1.00	5.31±1.51	<0.001S

Table 2: Comparative statistics among HbA1C ≤ 8.0 **and >8.0**

	HbA1C ≤ 8.0(N=48)	HbA1C > 8.0(N=52)	**P-value**
Male : Female	25:23 (52.08% vs 47.91%)	31:21 (59.61% vs 40.38%)	
Age	54.14±15.27	50.38±12.07	<0.001S
BSF (mg/dl)	139.88±36.73	245.75±91.29	<0.001S
BSPP (mg/dl)	231.50±64.55	312.50±88.39	<0.001S
HbA1C (%)	6.40±0.86	10.59±1.40	<0.001S
CRP (mg/dl)	11.625±9.03	49.06±35.32	<0.001S
ACR (mg/L)	35.44±30.04	69.53±60.38	<0.001S
Urea (mg/dl)	34.11±26.86	40.44±24.06	0.217NS
Creatinine (mg/dl)	0.93±0.70	1.13±0.79	0.185NS
Uric Acid (mg/dl)	4.85±1.00	5.34±1.87	0.011S

Table 3: Correlation coefficient among Variables

Group	**r value**	**P value**
HbA1C v/s CRP	0.539	<0.001S
HbA1C v/s MAU	0.237	0.017
CRP v/s ACR	0.325	<0.001S
HbA1C v/s ACR	0.18	<0.001S

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