European Journal of Molecular & Clinical Medicine (EJMCM)
ISSN: 2515-8260 Volume 08, Issue 04, 2021

Original research article

Study Of Diabetic Nephropathy In Relation With cystatin-C Level And Inflammatory Markers (hs-CRP) In Type-2 Diabetes mellitus Patients

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Abstract

India leads the world with largest number of diabetic population which is expected to rise around 69.9 million by 2025 unless urgent preventive steps are taken. Prolonged uncontrolled diabetes is associated with complications such as nephropathy, cardiovascular disease, retinopathy etc . Diabetic nephropathy is the largest single cause of end-stage renal disease. New pathways involved in the development and progression of diabetic kidney disease have been elucidated such as cystatin-C (a surrogate endogenous marker for estimating early decline in GFR in diabetes which can help to detect early kidney injury) and inflammatory markers including hs-CRP, TNF-α, IL-6 etc

Aim : To evaluate the association of cystatin-C levels and Inflammatory marker(hs-CRP) in diabetic nephropathy patients of India with type-2 diabetes mellitus.

Materials and Methods : A total of 150 samples from type-2 diabetes mellitus patients with diabetic nephropathy were collected from SBIMS hospital, Bhilai (C.G). Serum will be separated by centrifugation. Parameters will be estimated by the following methods- a) Estimation of serum cystatin-C by Immunoturbidimetry. b) Estimation of serum hs-CRP by ELISA kit method.

Result and Observations : In the study out of 150 patients, 47 were females and 103 were males. All the patients having mean age of 53.16 ± 9.49 years. Mean Cystatin-C level among males was 2.85 ± 1.96. and that of in females was 2.44 ± 1.69. similarly Mean hs-CRP level among males was 8.10 ± 1.09 and that of in females was 7.47 ± 1.52. The correlation between Cystatin-C level and inflammatory marker hs-CRP was highly positively correlated among type 2-Diabetic Patients with Diabetic Nephropathy.

Keywords : hs-CRP, cystatin-C, TNF-α, IL-6

Introduction

Diabetes is a common global disease. India leads the world with largest number of diabetic subjects. According to the Diabetes Atlas 2006 published by the International Diabetes Federation, the number of people with diabetes in India currently around 40.9 million is expected to rise to 69.9 million by 2025 unless urgent preventive steps are taken. [1,2,3] .

Prolonged uncontrolled diabetes is associated with complications such as **nephropathy**, retinopathy, neuropathy, cardiomyopathy, vasculopathy and atherosclerosis.[4]
Diabetic nephropathy is the largest single cause of end-stage renal disease.[5] It is a multistage clinical syndrome with an acquired sclerotic injury associated with thickening of the glomerular basement membrane and mesangial expansion with progression into glomerulosclerosis, tubular necrosis and intestinal fibrosis which ultimately leads to renal failure.[6]

The overall prevalence of microalbuminuria & macroalbuminuria in patients with type 2 diabetes mellitus is 25% (13-27) & 14% (5-48) respectively.[7] Studies based in southern India have estimated that the current prevalence of overt nephropathy is 2.2% and of microalbuminiroa is 26.9%.[8]

In recent years, new pathways involved in the development and progression of diabetic kidney disease have been elucidated. Among several biomarkers, Cystatin-C as a surrogate endogenous marker for estimating early decline in GFR in diabetes has been proposed to be a promising marker which can help to detect early kidney injury.[9] Cystatin-C is a low molecular weight non glycosylated protein, which is produced by all nucleated cells in the body. It is removed from the blood stream and freely filtered by the glomerular membrane in the kidney and is not secreted by renal tubules & is completely reabsorbed by the tubules.[10] Several recent studies have also shown that patients with type 2 DM and overt nephropathy exhibit high levels of diverse acute phase markers of inflammation, including high senstivity C-reactive protein (CRP), tumor necrosis factor (TNF-α), fibrinogen, and IL-6.[11,12,13]

High sensitive C-reactive protein (hs-CRP) is considered earlier as the inflammatory biomarker and has been reported to be associated with diabetes. It is normally present as a trace constituent of serum or plasma at levels less than 0.3 mg/dl.[14]
In contrast, the relationships between low grade inflammation and diabetic microangiopathy are still unclear. However, most studies have reported an increase in acute-phase markers in patients with nephropathy and also in patients with microalbuminuria.[15,16]Accumulated data have emphasized the critical role of kidney injury & inflammation in the pathogenesis of diabetic nephropathy.

The present study has undertaken to evaluate the association of cystatin-C levels and Inflammatory markers (hs-CRP) in diabetic nephropathy patients of with type-2 diabetes mellitus which in turn will help in the early intervention and management of diabetic nephropathy cases.

AIM AND OBJECTIVES

AIM:
To evaluate the association of cystatin-C levels and Inflammatory marker(hs-CRP) in diabetic nephropathy patients of India with type-2 diabetes mellitus.

OBJECTIVES:
1. To compare the serum levels of cystatin-C and Inflammatory markers (hs-CRP) in diabetic nephropathy patients with type-2 diabetic mellitus.
2. Interpretation and assessment of renal function.

MATERIALS AND METHODS

STUDY DESIGN:

It is an observational type of study.
The Biochemical investigations are done in the Department of Biochemistry, Shri Shankaracharya Institute of Medical Sciences (SBIMS), Bhilai (chattisgarh).

SAMPLE SIZE:

- A total of 150 samples from type-2 diabetes mellitus patients with diabetic nephropathy were collected from SBIMS hospital, Bhilai and they were interviewed to obtain relevant data.

- Sample size was calculated by using given below formula:

$$N = \frac{z2(5\%) * p * q}{e2}$$

= 73.68 where: n=Sample size

z =level of significance (1.96)
P = Prevalence (6.04%)
q = 1-p
e = error (5%)

Note: An Indian study has identified the prevalence of diabetes mellitus in Indian population as 6.04.[19]

A study was out for a period of two years from the commencement of the study.

SELECTION CRITERIA OF PATIENTS:

All patients suffering from type-2 diabetes having diabetic nephropathy will be diagnosed and confirmed by physician with FBS and PPBS according to American Diabetes Association criteria (FBS ≥126 mg/dl & 2 hour PPBS ≥ 200 mg/dl) and micro albuminuria .

INCLUSION CRITERIA:

1. Age 30-80 years
2. Patients of both genders
3. Type-2 diabetes mellitus patients with diabetic nephropathy

EXCLUSION CRITERIA:

1. Acute inflammatory conditions (trauma, thermal injury, surgery, pancreatitis and inflammatory bowel disease).
2. Patients taking insulin therapy.
3. Pregnancy.
4. Cardiac patients.
5. Patients who are diagnosed with any malignancy.
6. Patients with other chronic infections.
7. Patients with any endocrine disorders.

METHODOLOGY

After obtaining informed consent, about 5ml of fasting venous blood samples will be drawn under aseptic precautions into a sterile bulb from selected subjects. Serum will be separated by centrifugation. Parameters will be estimated by the following methods:

1. **Estimation of serum cystatin-C by Immunoturbidimetry:[17]**
2. **Estimation of serum hs-CRP by ELISA kit method:[18]**
3. **Estimation of Serum Creatinine by Jaffe's Method:[20]**
4. **Estimation of urinary micro albumin by turbidimetric Method: [17]**

STATISTICAL ANALYSIS:

Results will be subjected for appropriate statistical analysis:

- T test will be used for group wise comparison of biomarkers.
- Co- relation analysis will be used to measure the relationship between the biomarkers.
- Diagnostic validity test such as specificity, sensitivity, positive predictive value, negative predictive value will be used to assess the utility of these parameters as biomarkers in diabetic nephropathy and to differentiate type-2 diabetic cases from healthy controls.

RESULT AND OBSERVATIONS

Statistical Analysis:

Table 1(a)

Age wise distribution of study subjests		
Age group	frequency	Persent
31-40	17	11.3
41-50	43	28.7
51-61	55	36.7
61-70	35	23.3
Total	150	100.0
Mean ± S.D = 53.16 ± 9.49		

Table 1(b)

Sex					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	F	47	31.3	31.3	31.3
	M	103	68.7	68.7	100.0
	Total	150	100.0	100.0	

Table 1 (a) and (b) shows gender and age wise distribution of study subjects. There was total of 150 individuals included in study among them 103(68.7%) were males and 47(31.3%) were females. Mean age of all subjects was 53.16 years with standard deviation 9.49.

Table 2:

Testing whether mean difference of FBS, Cystatin C, M_Albumin and Hs_CRP between Gender					
	Gender	Mean	Standard Deviation	T Value	P Value
FBS	Male	213.17	38.50	0.116	0.908
	Female	212.34	43.92		
	Female	2.82	0.35		
Cystatin C	Male	2.85	1.69	1.468	0.144
	Female	2.44	1.40		
M_Albumin	Male	153.89	67.93	1.584	0.115
	Female	136.09	53.86		
Hs_CRP	Male	8.10	2.09	1.860	0.065
	Female	7.47	1.52		

Table 2:After applying a T test for testing the mean difference for FBS, Cystatin C, M_Albumin and Hs_CRP between gender showing that p value was greater than 0.05 for all that indicate there is not a statistically significant difference between gender on FBS, Cystatin C, M_Albumin and Hs_CRP.

Scatter Plots

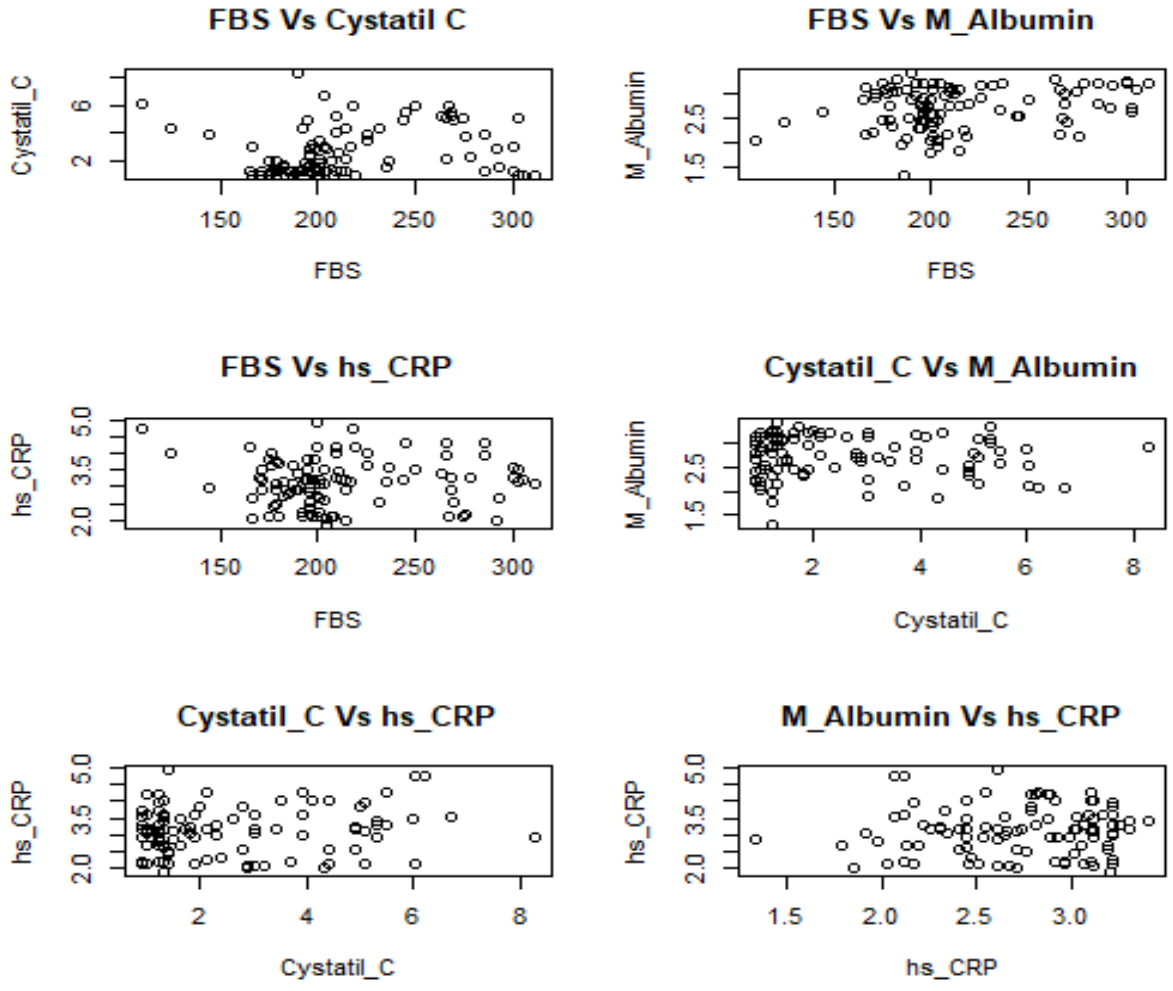

Table 3(a)

Correlation (Overall)				
	FBS	Cystatin C	M_Albumin	Hs_CRP
FBS	1	0.169	0.187	0.063
Cystatin C		1	0.657	0.638
M_Albumin			1	0.7
Hs_CRP				1

Table 3(a) shows that correlation between different laboratorical parameters. FBS is weak but positively correlated with Cystatin C (r = 0.169), M_Albumin (r = 0.187) and Hs_CRP (r = 0.063). There is moderate positive correlation of Cystatin C with M_Albumin (r=0.657) and Hs_CRP (r=0.638). Also, M_Albumin and Hs_CRP are moderate positively correlated (r =0.7). This indicate that the value of Hs_CRP increase with increasing in the value of Cystatin C.

Table 3(b)

Correlation (Female)				
	FBS	Cystatin C	M_Albumin	Hs_CRP
FBS	1	0.157	0.051	-0.0331
Cystatin C		1	0.604	0.347
M_Albumin			1	0.582
Hs_CRP				1

Table 3(b) shows that correlation between different laboratorical parameters in females. In females FBS is weak positively correlated with Cystatin C (r = 0.157), M_Albumin (r = 0.051) and weak negatively correlated with Hs_CRP (r = -0.0331). There is moderate positive correlation of Cystatin C in females with M_Albumin (r=0.604) and Hs_CRP (r=0.347). Also, M_Albumin and Hs_CRP are moderate positively correlated (r =0.582) in females.
This indicate that in females the value of Hs_CRP increase with increasing in the value of Cystatin C.

Table 3(c)

Correlation (Male)				
	FBS	Cystatin C	M_Albumin	Hs_CRP
FBS	1	0.177	0.246	0.211
Cystatin C		1	0.804	0.734
M_Albumin			1	0.745
Hs_CRP				1

Table 3(c) shows that correlation between different laboratorical parameters in males. In males FBS is weak positively correlated with Cystatin C (r = 0.177), M_Albumin (r = 0.246) and Hs_CRP (r =0.211). In males there is strong positive correlation of Cystatin C with M_Albumin (r=0.804) and Hs_CRP (r=0.734). Also, M_Albumin and Hs_CRP are strong positively correlated (r =0.745) in males.
This indicate that in males the value of Hs_CRP increase with increasing in the value of Cystatin C.

DISCUSSION AND CONCLUSION

150 type II DM patients with nephropathy with the age group 31 years and above admitted to shrishankaracharya institute of medical sciences and hospital were consider for the study.

There is limited data on type 2 DM nephropathy in Asia in order to find an easier method for detection of diabetic nephropathy as a screening method of diabetic nephropathy. We tried to find a relation between Hs-CRP, M albumin and TMF-alpha as a marker of diabetic nephropathy.

- Age Wise Distribution:

In this study mean age were 53.16 ± 9.49. Also the maximum 60% of the patients were more than 5% years age group. Also 13.3% of the patient were generation age group.

In a study done by chan-heejung et al. the mean age was 52.5 ± 10.0 year. Also EunHee Sim et al. found that the mean age was 57.5 ± 7.6 years. Similarly Swati Soni and Co-authors found that the mean age was 54.26 ± 8.11 years.

- Gender Wise Distribution:

In this study majority of the patients were belongs to the male was 68.7% and 31.3% of the female patients.

In a study done by Chan Hee Jung et al. was found that 65.4% of the male & 34.6% of the female. Also Sah JP et al. studied 89 patients out of then 51 were male and 38 were female. Similarly Vinod Kumar and co-authors were found that 150 (67.8%) were male and 71 (32.2%) were female.

- Fast in Blood Suger (FBS):

In this study the mean value of FBS was 213.17% ± 38.50 in male patients and 212.34 ± 43.92 in female patients. Also found that there is not a statistically significant difference between gender in FBS value.

In a study done by Vinod Kumar and Co-authors was found that the mean FBS value was 134.4 ± 51.5 but there is not a statistically difference between study subjects.

- Blood Urea:

In this study the mean value of B-Urea was 79.17 ± 27.41 in male patients and 77.49 ± 29.12 in female patients. Also found that there is not a statistically significant different between gender on B-Urea value.

- Serum Creatinine:

In this study was found that the average value of the serum creatinine was 2.95 ± 1.34 and 2.99 ± 1.18 in male and female respectively. Also stated that there is not a statistically significant difference between gender in serum creatinine (P value was 0.871).

In a study done by Chan Hee Jung et al. was found 1.0 ± 0.4 in the study participants. Also studied done by Sah JP and Co-authors ware found there is not a statistically significant different between gender in serum creatinine value. similarly Fun Hee Sim and at al. was found 0.76 ± 0.16 in diabetes patients. Also stated that there is no statistically significant in the study participants. Similar results were found in Vinod Kumar and Co-authors study, the average value of serum creatinine in Diabetic Nephropathy patients was 2.09 ± 1.57.

- Uric Acid:

In this study found that the UA mean value was 8.76 ± 0.89 in male participants and 8.95 ± 0.95 were female participants. Also shows that the there is not statistically significant mean difference between gender in UA.

Similarly Fun Hee Sim et al. study was found uric acid average value was 5.3 ± 1.5 and there is significant difference between study participant with normal glucose, diabetes and diabetes patients. Also Gupta S and friends found that the average value of Uric Acid was 8.55 ± 0.7 and also statistically significant difference between Diabetic Nephropathy and without diabetic nephropathy patients. In a study done by Kumai Nozomu and at al. were found 5.4 ± 1.3 average value of Uric Acid in the study participants.

- **Total Pro (TP):**

In this study was found that the average value of T-Pro 5.15 ± 0.98 were male and 5.33 ± 0.86 were female. Also found that there is not a statistically significant difference between gender as per mean value of T-Pro.

- **Serum Albumin**:

In this study was found that the average value of Serum Albumin 2.97 ± 0.46 were male and 2.82 ± 0.35 were female. Also found that there is not a statistically significant difference between gender as per mean value of Serum Albumin.

- Cystatin C:

In this study was found that the average value of Cystatin C 2.85 ± 1.69 were male and 2.44 ± 1.40 were female. Also found that there is not a statistically significant difference between gender as per mean value of Cystatin C.

Micro Albumin (M Albumin):

In this study we found that the average value of M Albumin 153.89 ± 67.93 were male and 136.09 ± 53.86 were female. Also found that there is not a statistically significant difference between gender as per mean value of M Albumin.

High sanctity C Reactive Protein (Hs CRP):

In this study was found that the average value of Hs CRP 8.10 ± 2.09 were male and 7.47 ± 1.52 were female. Also found that there is not a statistically significant difference between gender as per mean value of Hs CRP.

- Tumour Necrosis Factor Alpha (TNF Alpha):

In this study was found that the average value of TNF Alpha 35.04 ± 10.55 were male and 34.40 ± 9.15 were female. Also found that there is not a statistically significant difference between gender as per mean value of TNF Alpha.

Conclusion : hs-CRP is significantly positive correlated with UAE and Cystatin C. hs-CRP may play an important role that contributes to the progression of diabetic nephropathy.

Conflicts of Interest : None
Acknowledgement : None
Funding : None

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