DOI: https://doi.org/10.53350/pjmhs2216363

ORIGINAL ARTICLE

Association between Cystatin C, CRP in patients of Diabetic Nephropathy with Type 2 Diabetes Mellitus

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ABSTRACT

Background: Both developed and developing countries are facing the threat of end stage renal disease mainly caused by diabetes mellitus type 2.
Aim: To see an association between levels of Cystatin C and CRP in diabetic nephropathy patients.
Study design: Randomized controlled trial.
Methodology: A total of 285 patients confirmed Type 2 diabetes mellitus were inducted in study. Patients were subjected to standard interview questionnaire regarding age, duration and complications of diabetes. High sensitive latex enhanced immunoturbidimetric method was used to measure the serum levels of Cystatin C and CRP. High density lipoproteins (HDL), Low density lipoprotein (LDL), total cholesterol and glycosylated Hemoglobin (HBA1C) levels were also measured and interpreted. Doppler ultrasound was performed for structural evaluation of renal functions. SPSS 25.0 was used to analyze the whole data. The difference between all the groups was analyzed using One-Way Analysis of Variance (ANOVA).
Results: There was no statically Significant difference among age, sex, blood pressure, HDL, LDL, TC, and HBA1C levels among patients of all 3 groups, whereas CRP and Cystatin C levels were found to be raised with patients of moderate to severe nephropathy.
Conclusion: It was concluded that albumin to creatinine ratio (ACR), serum Cystatin C and CRP are potential biomarkers of disease progression in patients with diabetic nephropathy.
Keywords: Hyperglycemia, Albumin to Creatinine ratio, serum Cystatin-C, CRP and Nephropathy.

INTRODUCTION

Both developed and developing countries are facing the threat of end stage renal disease mainly caused by diabetes mellitus type-2. Clinically diabetic nephropathy is characterized by presence of microalbuminuria, however there is no strict criteria to label proteinuria as a significant sign of progression of diabetic nephropathy[1].

Recent studies had proved that all diabetic nephropathy patients showed microalbuminuria to some extent. Therefore physicians should be aware of clinical significance of proteinuria along with pathological changes that occurred during the progression of diabetic nephropathy[2].

Some studies had pointed toward the superimposed non diabetic renal diseases over diabetic renal lesions therefore biopsy and pathological changes are necessary to confirm these cases.[3] Glomerular lesions are the most prominent and significant changes that can be seen in patients with diabetic nephropathy. Basement membrane thickening and diffuse mesangial expansions are microscopic features of these glomerular changes[4]. Thickening of glomerular basement membrane can be observed 2-8 years after onset of type 2 diabetes mellitus. Most of studies are concerned with glomerular lesions but extra glomerular lesions are common as well. Tubulointerstitial fibrosis, and tubular atrophy are also common among patients of diabetic nephropathy[5].

Diabetes mellitus causes micro-angiopathy so both efferent and afferent arterioles have hyalinosis. Glomerular endothelial injury is also widely recognized pathology of diabetic nephropathy these days. Diabetic nephropathy is most common complication of type 2 diabetes[4,5]. Diabetic induced nephropathy is the leading causes of end stage renal disease. Diabetic nephropathy is the public health disaster with far reaching consequences on socioeconomic, physical and mental health. Diabetes prevalence in Asia is increasing as time passes on with concurrent increase in ageing population which is imposing a huge burden on economies of Asian developing countries.[6] Therefore it is imperative to investigate and explore prognostic, disease progression markers so as to rescue the healthcare system of developing countries.

Our study investigated the role of Cystatin-C in diabetic nephropathy Cystatin-C belongs to family of proteinase inhibitors. It is mainly present in urine, semen, colostrum and cerebrospinal fluid and mainly produced by nucleated cells. Recent studies had showed higher levels of Cystatin-C are poorly associated with diabetic retinopathy and age related macular changes[7.7] Our study also explored the C reactive protein levels (CRP) among patients of diabetic nephropathy. Active infection or inflammation produces CRP which is an acute phase reactant. It is mainly involved in innate immunity. High levels of CRP concerned with acute inflammation are indicative of endothelial injury in kidneys thus leading to diabetic retinopathy[8].

The objective of the study was to see an association between levels of Cystatin C and CRP in diabetic nephropathy patients**.**

METHODOLOGY

After permission from the Institutional Ethical Review Board a total of 285 patients of diabetes visited from 2016 to 2021 at Fazilat Amin Hospital Gujrat were inducted in this study. Simple random sampling was performed to collect samples from these patients. Following patients were excluded from study: 1.Any other complication of diabetes or type 1 diabetes. 2. Patients with diagnosed case of end stage renal diseases concurrent with diabetes due to other causes. 3. Other systemic complications of diabetes.

Diabetes standards of WHO (199) and 2012 criteria of American diabetic association was used to diagnose type 2

Received on 13-10-2019
Accepted on 03-03-2022

diabetes in patients. Patients were subjected to standard interview questionnaire regarding age, duration and complications of diabetes. Digital automatic blood pressure measuring device was used to measure blood pressure after patient was relax and seated for 5 minutes. High sensitive latex enhanced immunoturbidimetric method was used to measure the serum levels of Cystatin C and CRP. High density lipoproteins (HDL), Low density lipoprotein (LDL), total cholesterol and glycosylated Hemoglobin (HBA1C) levels were also measured and interpreted.

Nephropathy evaluation: Doppler ultrasound (US) for renal function and structural changes was performed on each visit to monitor the progression of disease. Albumin to creatinine ratio (ACR) was measured by urine dipstick in spot urine on each visits. Patients were grouped into 3 groups. Group 1 normal or mild elevated ACR, Group 2 Moderately increased ACR, Group 3 severely increased ACR.

Statistical analysis: SPSS 25.0 statistical software was used to analyze the whole data. The quantitative variables were summarized using mean $\pm$ SD. The difference between all the groups was analyzed using One-Way Analysis of Variance (ANOVA) which was followed by Tukey's Post Hoc correction for multiple comparisons. A value of $P \leq 0.05$ was considered statistically significant.

RESULTS

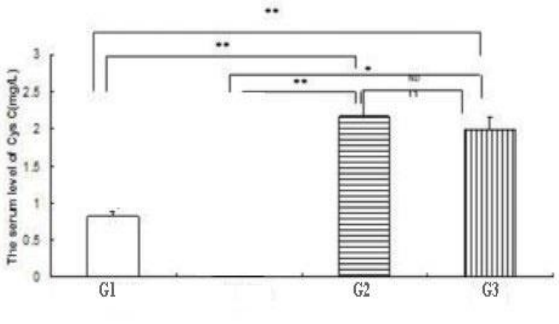
We inducted 285 patients and were divided into 3 groups. All 3 groups clinical date was represented in table-1. There was no statically Significant difference among age, sex, blood pressure, HDL, LDL, TC, and HBA1C levels among patients of all 3 groups.

Table-1: Clinical Characteristics of the population

Parameters	G1 (n=95)	G2 (n=110)	G3 (n=80)
AGE	51.1±9.11	53.7±6.21	55.1±10.07
M/F	30/65	45/65	55/25
SBP/DBP(mmHg)	130/91	132/95	135/85
Duration of diabetes(years)	12.1	12.7	13.5
HbA1c (%)	8.1	7.9	8.2
Total-cholesterol (mmol/L)	3.97±0.89	4.33±0.67[a]	4.61±1.03[a,b]
LDL- cholesterol (mmol/L)	2.95±0.81	4.31±0.77	4.61±1.01
hs-CRP(mg/L)(0-8)	2.95±1.92	5.12±2.03	5.42±1.31
CysC(mg/L)(0.51-1.09)	0.92±0.04	2.05±0.14	1.97±0.46
Albumin to creatinine ratio (ACR) (mg/g)	28.4±1.48	99.7±2.22	322±2.36

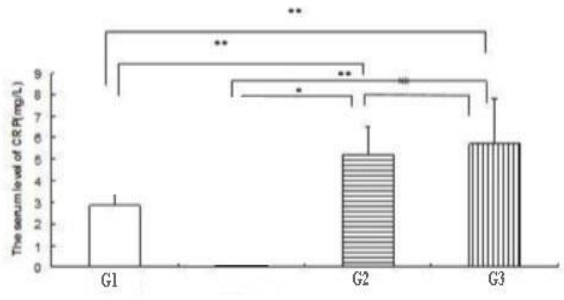
High sensitive latex enhanced immunoturbidmetric method was used to find serum levels of Cys C, among three groups. We found out that levels of Cys C was statistically significant (p=0.03) among all 3 groups. There was no difference of Cys C levels among normal or mild elevated ACR group. Whereas moderate and severely elevated ACR groups showed statistically significant difference (p=0.01) as shown in fig-1.

Fig-1: Serum Cystatin-C levels among all groups



CRP levels were also measured and found to be higher in moderate and severely elevated ACR group (p=0.01) whereas no significant increase in levels of CRP was observed in mildly elevated ACR group as shown in fig-2.

Fig-2: Measured serum CRP levels among all groups.



DISCUSSION

Serum levels of Cystatin C and CRP are elevated in patients with moderate to severe ACR, which points toward the excessive inflammation and massive endothelial injury. So it can be said that diabetic retinopathy is not just an endothelial injury it's a neurovascular disorder[9].

Lysosomal and extracellular cysteine proteinases are inhibited by Cystatin C and its expressed by variety of cells in body such fibroblasts, pancreatic islets cells, endothelial cells and glial cells. The major site of Cystatin C expression in kidneys are glomerular cells which can lead to extensive scarring and malfunction of glomerular apparatus thus causing end stage renal disease[10].

Some studies had explored the role of Cystatin C in vessels remolding, neovascularization, and neuronal degenerative pathology. Similarly diabetic nephropathy results from extensive endothelial damage vascular remolding and inefficient filtration process[11].

The marker for chronic inflammation such as preclinical atherosclerosis, systemic endothelial dysfunction, arterial wall inflammation is CRP[12]. It's an acute phase reactant produced as a result of inflammation. Concurrent increase in CRP in patients with elevated levels of Cystatin C showed extensive inflammation is taking place and that might be causing the glomerular apparatus to becoming less responsive to filtration and consequently leading to end stage renal failure[13]. Doppler ultrasound is mainstay of investigating the structural abnormalities of diabetic retinopathy. However albumin to creatinine ratio is used to assess the renal function and degree of nephropathy[14].

Our study also explored the levels of albumin to creatinine ration and was found to be elevated in moderate to severe disease progression. The most reliable and recommended method of measuring albumin to creatinine is on spot urine. The gold standard of measuring the ACR is to collect 24 hour urine output. However we used ACR instead of 24 hours urine collection cause of state of hydration and convenience of patient. Historically mild to moderate increase in albumin is known as microalbuminuria[15]. Our study correlated the levels of albuminuria to Cystatin C and CRP with concurrent structural changes on Doppler ultrasound.

Limitations: Limitations included limited sample size, time frame, resources and financial constrains.

CONCLUSION

It was concluded that albumin to creatinine ratio (ACR), serum Cystatin C and CRP are potential biomarkers of disease progression in patients with diabetic nephropathy. Therefore further studies are needed to explore the underlying pathophysiological process governing these pathological changes due to elevated levels of these biomarkers.

Author's Contribution: QUA&RZB: Conceptualized the study, analyzed the data, and formulated the initial draft, **SB&US:** Contributed to the proof reading, **WUK&STN:** Collected data, **MZA, AUH&GH:** Contributed to the proof reading, **TL**: Contributed to the proof reading the manuscript for intellectual content.
Conflict of interest: None
Funding: None

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