Original Article

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The Pathogenic and Developmental Biomarker: High Sensitivity C-Reactive Protein in Early Diagnosis of Diabetic Nephropathy

Biochemistry Section

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ABSTRACT

Introduction: Type 2 Diabetes Mellitus (T2DM) is one of the chronic metabolic conditions leading to various complications and most commonly Diabetic Nephropathy (DN). DN imposes a major and unrecognised burden to the whole world in the management of health system and economy. High sensitivity C-Reactive Protein (hs-CRP), being an inflammatory marker, plays a pathogenic role in DN and found to be increased in diabetes patients.

Aim: To determine an earlier pathogenic marker for DN among T2DM patients.

Materials and Methods: This cross-sectional study was conducted among 92 clinically diagnosed T2DM patients. Written informed consent was obtained. Blood samples were collected and analysed for Fasting Blood Sugar (FBS), hs-CRP. Spot urine microalbumin creatinine ratio was estimated by immunoturbidimetry and Jaffe's method, respectively. Data was statistically analysed using student t-test and correlation between analytes was analysed using Pearson correlation.

Results: Mean serum hs-CRP value of diabetic subjects with microalbuminuria was 6.9±3.2 mg/L and diabetic subjects without microalbuminuria was 1.4±0.68 mg/L. There was a positive correlation between hs-CRP and Urine Albumin Creatinine Ratio (UACR) (r=0.47; p=0.02). There was also a positive correlation between hs-CRP and FBS levels and hs-CRP and duration of type 2 diabetes mellitus. The hs-CRP and UACR were elevated among type 2 diabetic patients. There was a positive correlation between hs-CRP and FBS and duration of diabetes mellitus.

Conclusion: As hyperglycaemia plays a critical pathogenic role in type 2 diabetes through the inflammatory pathway, hs-CRP may be suggested as a developmental biomarker of DN among T2DM patients in association with UACR.

Keywords: Diabetic kidney disease, Fasting blood sugar, Urine microalbumin creatinine ratio

INTRODUCTION

Type 2 Diabetes Mellitus is a metabolic disorder with hyperglycaemia which develops due to the defect in insulin secretion, insulin action or both [1]. The chronicity of hyperglycaemia leads to the long term damage and failure of various organ systems. Thus, T2DM is associated with macrovascular diseases (coronary artery diseases, cerebrovascular diseases and peripheral arterial diseases) as well as microvascular diseases (retinopathy, nephropathy and neuropathy) [2]. The estimated number of people with diabetes have increased by 88% from 246 million in 2006 [3] to 440 million in 2015 [4] and 463 million in 2019. Diabetes is a serious chronic condition associated with diffuse complications and an increased risk of premature death, imposing enormous financial pressure on national healthcare systems and national economies [5].

Death in people with diabetes is mainly due to diabetes-related complications rather than disease per se [6]. All the small blood vessels in organs like kidney, eyes and nerves get affected in almost 15 years. It is estimated that more than 20-40% of diabetic patients will develop Chronic Kidney Disease (CKD) depending on the population and a significant number may develop End Stage Kidney Disease (ESKD) requiring renal replacement therapies like renal transplant [7,8]. In patients with Type 2 DM, it is recommended to do screening to diagnose early and monitor yearly thereafter with more robust tests [9].

Chronic inflammation leads to insulin resistance and hence linked to hyperglycaemia. Hyperglycaemia induced inflammatory process increases the levels of pro-inflammatory proteins [10]. The activated leukocytes and endothelial cells produce IL-6, a pleiotropic inflammatory cytokine and CRP which is an acute phase plasma protein is synthesised by the liver and is a sensitive systemic biomarker of inflammation [11]. Excess glucose has been shown to be associated with about 15% of all deaths due to CVD and Diabetic Kidney Disease (DKD). [12] Short term increase in hyperglycaemia does not result in serious clinical complications. The duration and severity of hyperglycaemia is the major causative factor in initiating organ damage and in renal damage, the damage is best ascertained from proteinuria and glomerular damage [13]. The prolonged duration of uncontrolled hyperglycaemia induces advanced glycation end products which activates Nuclear Factor κB. The NFκB acts on the nucleus through transcription and releases cytokines. These cytokines are cytotoxic to glomerular and epithelial cells which induce direct renal damage [14]. The hyperglycaemia induced increase in inflammatory marker hs-CRP causing the promotion in renal inflammation leads to the DN [15].

Even though urine microalbumin creatinine ratio is a biomarker of the DN, but the nephropathy has already happened. The aim of the study was to observe the level of hs-CRP among type 2 diabetics as it plays a pathogenic role and to determine an early marker for DN in association with UACR.

MATERIALS AND METHODS

This cross-sectional study was conducted in a details of the Tertiary Care Centre for a period of eight months from January-August 2015. The study proposal was approved by the Institutional Ethical Committee No: NMC/IEC/07/11/014 and informed consent was obtained from all the study participants.

Inclusion Criteria

Ninety two clinically diagnosed T2DM patients of age group of 30-70 years were selected, as the prevalence of T2DM is common in this age group.

Exclusion Criteria

The subjects with history of any significant infectious diseases, trauma, malignancy, active immunological diseases, cardiovascular

diseases, tuberculosis, pregnancy or confounding factors for proteinuria such as severe uncontrolled hypertension or renal insufficiency were excluded from the study.

Detailed history was taken and physical examination was done for all subjects who were enrolled in the study. The study participants were divided into two groups, diabetics with microalbuminuria as cases and without microalbuminuria as controls.

Random urine samples were collected for the quantitation of albumin and creatinine. Urine microalbumin creatinine was estimated by immunoturbidimetry and Jaffe's method, respectively using the auto-analyser and the results were reported as urine albumin creatinine ratio (mg/g). The daily albumin excretion was assessed by using the concentration ratio of urine albumin to creatinine. The urine albumin value between 30-300 mg/g was defined as microalbuminuria. Presence of microalbuminuria was equated as a urinary albumin: creatinine ratio, which was based on the National kidney Foundation's Kidney disease Outcome Quality Initiative working group definition [16]. FBS values were estimated from the blood samples. The hs-CRP was estimated by immunonephelometry method using auto-analyser. Analytical sensitivity for hs-CRP in this assay system was 0.06 mg/L. The desirable level of hs-CRP is <1 mg/L.

STATISTICAL ANALYSIS

Statistical analyses were performed using SPSS. Continuous variables were expressed as mean±standard deviation. Data were analysed using independent t-test for their level of significance. Chi-square test was used to determine the association between hs-CRP and microalbuminuria, FBS and duration of diabetes mellitus. Correlation between variables was carried out using the Pearson correlation coefficient. The value of p<0.05 was taken as significant.

RESULTS

The present study enrolled 92 clinically diagnosed T2DM subjects. Out of 92 diabetic subjects, 48 subjects had microalbuminuria (cases), whereas 44 subjects were without microalbuminuria (controls). The mean of duration of type 2 diabetes was 6.7±3.04 years.

[Table/Fig-1] demonstrates the mean and standard deviation of various parameters among diabetic patients. [Table/Fig-2] demonstrates the increased hs-CRP in diabetics with microalbuminuria compared to diabetics without microalbuminuria. Mean UACR of diabetic patients with microalbuminuria was 128.6±53.2 mg/g and diabetic subjects without microalbuminuria was 9.4±5.6 mg/g. This difference was found to be statistically significant (p-value <0.001).

Parameters	Mean±SD
Total participants	92
Age (years)	48.59±12.54
Duration of T2DM (years)	6.7±3.04
BMI (kg/m^2)	25.33±3.74
FBS (mg/dL)	188.08±91.85
hs-CRP (mg/L)	5.11±3.09
UACR (mg/g)	96.32±65.78

[Table/Fig-1]: Demonstrates the mean and standard deviation of various parameters among diabetic patients.
The t-test has been used to evaluate the p value

Total number of cases n=92	Raised hs-CRP (%)	Normal hs-CRP (%)	p-value
Diabetics with microalbuminuria (n=48)	39 (81.2 %)	9 (18.7%)	<0.0001
Diabetics without microalbuminuria (n=44)	8 (18.1%)	36 (81.8%)	

[Table/Fig-2]: Demonstrates hs-CRP in diabetics with microalbuminuria compared to diabetics without microalbuminuria.
The t-test has been used to evaluate the p value

[Table/Fig-3] depicts the mean value of UACR and hs-CRP of diabetic subjects with and without microalbuminuria. Mean serum hs-CRP value of diabetic subjects with microalbuminuria was 6.9±3.2 mg/L and diabetic subjects without microalbuminuria was 1.4±0.68 mg/L. This difference was also found to be statistically significant (p-value <0.001). It was found that out of 48 subjects with microalbuminuria 39 (81.2%) patients had associated increase of hs-CRP level (p-value <0.001).

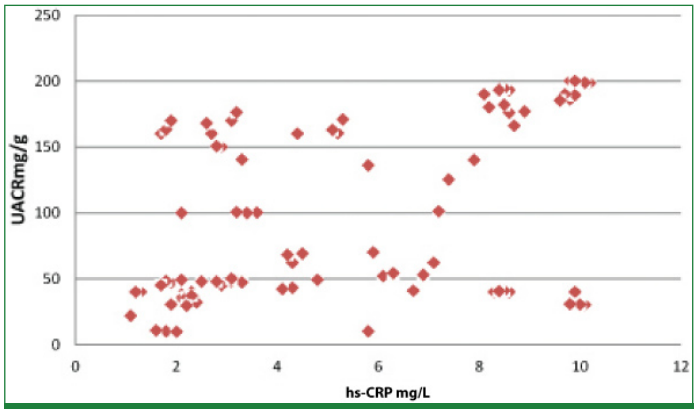
Variables	Diabetics with microalbuminuria (n=48) (Mean±SD)	Diabetics without microalbuminuria (n=44) (Mean±SD)	p-value
Urine albumin creatinine ratio (mg/g)	128.6±53.2	9.4±5.6	<0.001
hs-CRP (mg/L)	6.9±3.2	1.4±0.68	<0.001

[Table/Fig-3]: Depicts the mean value of UACR and hs-CRP of diabetic subjects with and without microalbuminuria.

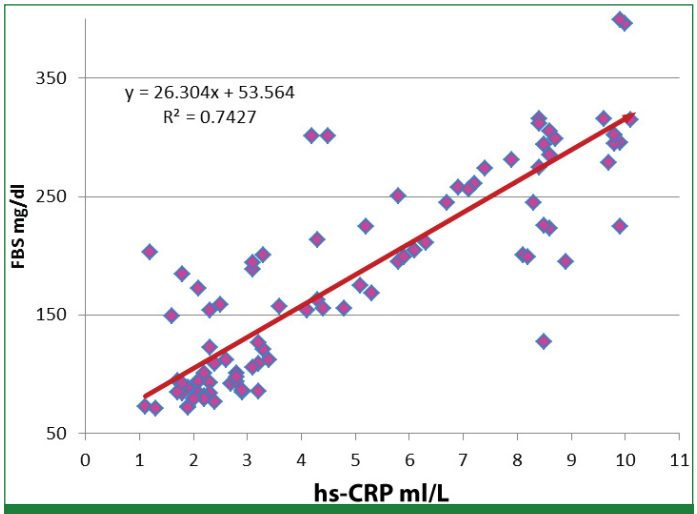
[Table/Fig-4] depicts the association between hs-CRP and UACR, FBS and duration of T2DM in diabetic subjects. Correlation between variables was carried out using Pearson correlation coefficient which showed a positive correlation between hs-CRP and UACR (r = 0.47; p=0.02) which is shown in [Table/Fig-5] which demonstrates the correlation between hs-CRP and urine albumin creatinine ratio level. There was also positive correlation between hs-CRP and blood sugar and hs-CRP and duration of diabetes mellitus which are demonstrated in [Table/Fig-6.7], respectively.

Parameters	hs-CRP (r value)
UACR	0.47
FBS	0.742
Duration of T2DM	0.62

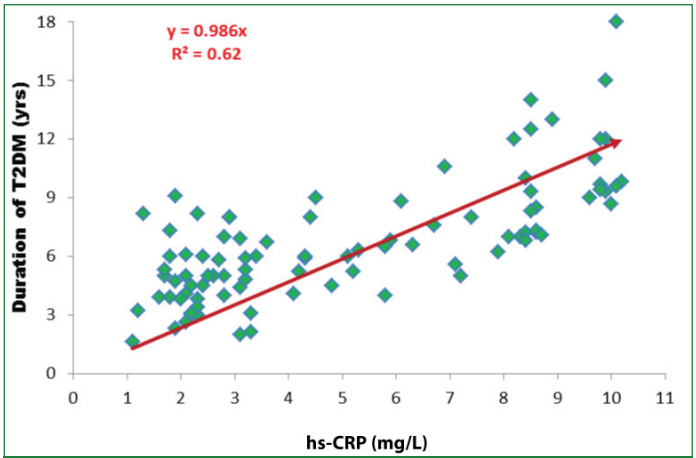
[Table/Fig-4]: Demonstrates the correlation between hs-CRP and other parameters.



[Table/Fig-5]: Demonstrates the correlation between hs-CRP and urine albumin craetinine ratio level.



[Table/Fig-6]: Demonstrates the correlation between hs-CRP and Blood sugar level.



[Table/Fig-7]: Demonstrates the correlation between hs-CRP and Duration of diabetes mellitus.

DISCUSSION

The study was conducted to identify an earlier biomarker for DN among T2DM patients. Although urinary albumin is recognised as an early marker of DN, but significant glomerular damage might have already occurred when albumin appears in urine [9]. As albuminuria has certain limitations the need for more reliable serum biomarker with high sensitivity is needed to diagnose the DN at the earliest during the developmental stage [17].

Among type 2 diabetics, urinary albumin increase by 1.02 mg/24 h (95% CI 1.01-.27) for each increase in CRP of 1 mg/L over ten years of follow-up has been reported for the first time by Stehouwer CD et al., which correlates with the findings of the present study [18]. Mohd IK et al., suggested the role of inflammation in pathogenesis of microalbuminuria and reported a poor correlation between hs-CRP and microalbuminuria (p=0.002) as a contrary finding to the present study [19]. A positive association was shown in an animal study, where the CRP administration to rats resulted in endothelial dysfunction and impaired vascular activity by inhibiting endothelial nitric oxide synthase [20]. The decreased production of nitic oxide promotes vasoconstriction, platelet activation, leucocyte adherence, impaired coagulation and vascular inflammation [21]. Immunoregulatory function of hs-CRP induces the leukocyte reactivity, complement fixation, modulation of platelet activation and clearance of cellular debris and can directly change glomerular function and thereby influences the development of microalbuminuria. Thus the increased production of inflammatory cytokines during the pathogenesis of diabetes mellitus will be the causative factor for increase of both hs-CRP and microalbuminuria [22]. The study conducted by Hayashino Y et al., among type 2 diabetics, a prospective cohort study, has reported that serum hs-CRP levels, independent of the possible confounders, were associated with a risk of developing DN is correlating with the findings of the present study and strengthens the significance of hs-CRP in the diagnostic aspect [23]. In a study by Saraheimo M et al., among type 1 diabetics also a similar association was observed between CRP levels and DN [24]. CRP levels high in type 2 diabetes patients with microalbuminuria compared with those with normoalbuminuria has been reported in a study by Navarro JF et al., [25]. Xiaohua W et al., has also suggested the role and association of hs-CRP in the diagnosis of DN at the early stage in a study done among type 2 diabetics [15]. The study on inflammatory markers by Waheed P et al., showed the relationship between CRP and low grade inflammation in diabetic subjects [26].

The hs-CRP itself is induced by high levels of glucose, promoting renal inflammation. So, hs-CRP may serve as an inflammatory mediator of high glucose levels to promote the diabetic renal inflammation [27]. In the present study, there is increased hs-CRP among diabetics with moicroalbuminuria and there is a positive correlation between hs-CRP and hyperglycaemia and its duration also. The same has been evidenced in a study among type 2 diabetics by Kaefer M et al., and showed significant correlation between hs-CRP and FBS [28]. A study by King DE et al., on hs-CRP and glycated haemoglobin also indicated the relationship between hs-CRP and glycaemic control [29]. In uncontrolled T2DM patients, hyperglycaemia as an associated factor with the increased serum CRP levels has been reported by Lima LM et al., [30]. Among the urban North Indian type 2 diabetics, Mahajan A et al., found a relationship between CRP and hyperglycaemia similar to the index study [31].

The progression and regression of kidney disease in T2DM is highly variable as it is usually diagnosed with the secondary disorders, the onset of which is unrecorded. The UKPDS study reported microalbuminuria and reduced Glomerular Filtertion Rate (GFR) in 38% and 29% patients, respectively after a median follow-up of 15 years. In terms of progression, the same study reported a change from microalbuminuria to macroalbuminuria and End Stage Kidney Disease (ESKD) at 2.8% and 2.3% per year, respectively [32]. Study on Pima Indians reported that macroalbuminuria was 50% during the median follow-up of 20 years. Also, a gradual loss of kidney damage with time was noticed as 7.3% patients were diagnosed with microalbuminuria at the onset, 17.3% at 5 years, 24.9% at 10 years and 28% at 15 years. Epidemiological studies in western and Pima Indian population also suggests that the prevalence of overt nephropathy is about 20-25% in patients with type 2 diabetes mellitus, depending solely on the duration since the onset of disease [33]. Treatment is not effective for the patients with advanced renal lesions when compared to the patients at the earlier stages of the disease. For them UACR may be a marker rather than the predictor of DN. Thus, it may be better to identify normoalbuminuric patients at increased DN risk in order to select those at the early stages still amenable to aggressive intervention strategies such as strict glycaemic control [34]. The study by Mohd IK et al., has also suggested the significance of early diagnosis of renal damage and control of hyperglycaemia, for the prevention of diabetes related complications [19]. Thus, screening of hs-CRP in type 2 diabetic patients could identify the renal vascular complications and further development of DN at the earliest.

Limitation(s)

The hs-CRP alone is suggested as a complementary marker with UACR and the other markers of endothelial injury were not studied among the cases due to the cost effective reasons is one of the limitations of the study. Even the study population is small in the study.

CONCLUSION(S)

In summary, there is a significant association between the level of serum hs-CRP and microalbuminuria in type 2 diabetes patients. There is also a positive correlation between hs-CRP and blood sugar level and duration of T2DM. Hence, the activation of inflammatory pathways by hyperglycaemia, which is indicated by increased hs-CRP can be used in the screening stage of DN. So the type 2 diabetic patients may be regularly monitored for the more sensitive and causative biomarker of nephropathy, hs-CRP and the confirmatory and early biomarker UACR to facilitate the early and definitive diagnosis of DN.

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