International Journal of Research in Medical Sciences
Rastogi A et al. Int J Res Med Sci. 2023 Nov;11(11):4136-4141
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012
DOI: https://dx.doi.org/10.18203/2320-6012.ijrms20233388

Original Research Article

Study of association between C-reactive protein and albuminuria in type 2 diabetes mellitus

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Received: 11 September 2023
Revised: 10 October 2023
Accepted: 11 October 2023

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ABSTRACT

Background: Inflammatory markers are excessively produced by adipocytes in T2DM due to obesity-induced dysregulation of adipocytes. Inflammation is recognised by elevated level of inflammatory markers like C-reactive protein. It has been reported that patients with nephropathy and those with albuminuria have higher levels of inflammatory markers.
Methods: Study design observational descriptive cross-sectional study. 150 subjects having age more than 30 years and less than 55 years diagnosed with type 2 diabetes mellitus were included. HbA1c, FPG, creatinine, urea, CRP, and albuminuria were analysed.
Results: A strong and statistically significant correlation was seen between serum CRP levels and albuminuria levels with a p value of 0.831 and a p value of 0.00. The study group was divided into two groups with normal CRP (n=40) and elevated CRP (n=110). Average values of all parameters showed a statistically significant increase in the group with abnormal CRP levels. In the ROC analysis, an area of .957 under the curve shows a very high predictive value of 15.5 mg/l of CRP for predicting albuminuria in patients of T2DM.
Conclusions: From our results, we have been able to establish a strong association as well as a predictive relationship between the level of CRP and albuminuria in T2DM. We have shown that serum CRP levels at a cut-off of 15.5 mg/l are a predictor of clinically significant albuminuria. This makes serum CRP level an effective screening tool for albuminuria.

Keywords: Albuminuria, C-reactive protein, Diabetic nephropathy, Microvascular complication, Type 2 diabetes mellitus

INTRODUCTION

Diabetes mellitus (DM) refers to a set of known metabolic conditions that can be caused by different genetics and environmental factors, which share the phenotype of hyperglycaemia. The prevalence of DM in India as of 2019 was 8.9% with an increase from 7.1% a decade before.[1] It affects almost 7.34% of the Western Uttar Pradesh population, according to 2020 data.[2] The diagnosis of diabetes mellitus can be broadly classified into two categories Insulin-dependent diabetes mellitus (T1DM) and Insulin Non-dependent Diabetes Mellitus (T2DM).[3] T1DM is described by a near-total lack of insulin due to destruction of β-cells of the pancreas. β-cell destruction can be due to drugs, viruses, or auto-immunity.[4] It usually develops in children, teenagers, and young adults; however, it can happen at any age. T2DM is triggered by insulin resistance, and in previous literature, obesity, especially excessive abdominal fat called visceral fat, are the key reason for insulin

resistance.[5] In a person with insulin resistance, cells in their muscles and adipose tissue, don't respond well to normal or increased levels of insulin.[6] This is characterized by a decrease in the target cell response to insulin. Inflammatory cytokines and inflammatory markers are excessively produced by adipocytes in T2DM due to obesity-induced dysregulation of adipocytes.[7] Type 2 diabetes mellitus has emerged as one of the most critical chronic public health problems and is a rising reason of morbidity and mortality. T2DM is the leading cause of various micro and macro vascular complications.[8] Micro-vascular complications include neuropathy, retinopathy, and nephropathy. Macro-vascular complications consist of coronary artery disease (CAD), cardiomyopathy, cerebrovascular diseases, peripheral artery disease, etc.[9] Several epidemiological and clinical researches have proven that microalbuminuria is a sign of vascular damage. Microalbuminuria (MAU) is also a proven marker of diabetic nephropathy.[10] Low-grade inflammations is linked to T2DM, inflammation is recognized by elevated level of inflammatory markers like C-reactive protein, interleukin-6, and tumor necrosis factor-α.[11] The C-reactive protein is produced by hepatocytes in response to cytokines secreted by macrophages and adipocytes.[12] CRP is a sensitive marker of low-grade inflammation. It has been reported that raised serum CRP levels are related to complications of diabetes mellitus. The levels of CRP in the plasma relate to the complication of diabetes mellitus and the degree of glycaemic control.[13] The effects of CRP on endothelial cell activation and adhesion molecule expression result in endothelial dysfunction.[12] It has been reported that patients with nephropathy and those with microalbuminuria have higher levels of acute phase markers such as CRP.[14] As CRP has been reported with renal risk factors, such as it could play a predictive role in renal events in subjects with T2DM.[15] Taking into account the above facts, higher serum CRP levels could be valuable as an initial indicator of renal function derangement in subjects with T2DM.

The objective of the study to establish association between the levels of CRP and albuminuria among study subjects.

METHODS

The study was conducted at a tertiary care hospital of western Uttar Pradesh. It was designed as a cross sectional observational descriptive study. The sample size 150 of the study was based on the prevalence of T2DM in India. Ethical clearance was obtained from the ethical committee of the hospital.

Subjects selection

The subjects having age more than 30 years and less than 55 years diagnosed with type 2 diabetes mellitus (According to American Diabetes association criteria) by the Department of Medicine were included in this study group after obtaining an informed consent. Subjects having history of chronic renal diseases, coronary artery diseases, any type of known cases of any active infection, chronic inflammatory diseases such as cancer, tuberculosis, patients with the history of use of nephrotoxic drugs, and pregnant or lactating women were excluded.

Assessment of biochemical parameters

About 5 ml of blood sample was collected after 8 hours of fasting for the analysis of HbA1c, FPG, C-reactive protein, creatinine, and urea. Morning first void urine sample was collected in a urine container for the analysis of albuminuria. Serum was separated from blood by centrifugation at 5000RPM for 5 minutes. Estimation of HbA1c was done by high-performance liquid chromatography using the Bio Rad D10 system. Estimation of plasma glucose was done by hexokinase method, run on a cobas automatic analyzer and Glucose HK gen.[3] cobas kit. Estimation of C-reactive protein was done by turbidimetric immunoassay method, using the semiautomatic analyzer and the working reagent kit of Avecon. Creatinine estimation was done using the modified Jaffe's method, tests run on cobas automatic analyzer, and using the creatinine Jaffe's gen.2 cobas kit. Urea estimation was done using the urease method tests run on a Cobas automatic analyzer and using a urea/BUN cobas kit. The estimation of albuminuria was done by the pyrogallol red method, the test was performed on a semi-automatic analyzer and using a micro protein kit. Cut-off values of HbA1c, FPG, Serum CRP, Serum creatinine and Serum urea, were taken as <6%, 60-100 mg/dl, <6 mg/L, 0.5-1.2 mg/dl, and 15-45 mg/dl, respectively. For albuminuria a value of 200 mg/l was taken as cutoff in the first morning void sample.[16]

Statistical analyses

Data was analysed by Statistical Package for Social Sciences (SPSS-28). Different measuring tools of SPSS were used for data analysis, such as descriptive analysis of data, Spearman's correlation coefficient test was used for correlation of parameters and Receiver-Operating Characteristic (ROC) Analysis of CRP.

RESULTS

Analysis of demographic data

This study was included 150 patients diagnosed with T2DM, age group between 30 to 55 years. In 150 individuals, 79 (53%) females and 71 (47%) males. The male and female groups were classified into two age groups, the first age group from (30-44) age and the second age group from (45-55) age. In the first age group (30-44), 22 male individuals and 20 female individuals found, but in the second age group (45-55), 49 male individuals and 59 female individuals found (Table 1). Descriptive analysis of different biochemical parameters (Table 2).

Table 1: Analysis of demographic data.

Gender	Age groups	Count	Percentage*	Relative percentage**
Female	30-44	20	25	53
	45-55	59	75	
	Total	79	100	
Male	30-44	22	31	47
	45-55	49	69	
	Total	71	100	

*percentage estimation of counts for particular age group for male and female cases; **relative percentage estimation of counts for all males and females.

Table 2: Descriptive analysis of different biochemical parameters.

Biochemical parameters	Cases (n)	Range	Minimum	Maximum	Mean	Std. deviation
FPG	150	264.4	92.6	357.0	156.6	39.3
HbA1c	150	11.9	4.6	16.5	8.2	2.4
CRP	150	49.9	0.66	50.6	12.9	9.8
Albuminuria	150	1084.6	1.38	1086.0	123.8	144.3
Creatinine	150	2.40	0.51	2.91	0.8	0.3
Urea	150	69.0	15.0	84.0	33.1	12.5

Comparative analysis of biochemical parameters

In the study subjects, 40 subjects with normal CRP mean value was 3.3 mg/l with std. deviation of 1.6 mg/l, but 110 subjects with abnormal CRP mean value was found 16.4 mg/l with std. deviation of 9.3 mg/l. The difference in values of CRP in the two groups was statistically significant with a p value of 0.00. The mean values of Albuminuria, Serum creatinine, Serum urea, HbA1c, Fasting plasma glucose among the two groups was as such (Table 3). All parameters show an increase in value in the group with the abnormal high CRP levels as compared with the group having normal CRP levels. The difference between the two groups is statistically significant for all parameters (Table 3).

Table 3: Comparative analysis of biochemical parameters.

Biochemical parameters	Normal CRP group (n=40)	Abnormal CRP group (n=110)	p value
Albuminuria Mean±SD	28.9±28.2	158.2±153.9	p<0.001
Creatinine Mean±SD	0.7±0.2	0.9±0.3	p<0.001
Urea Mean±SD	30±8.5	34.2±13.5	p<0.001
Hba1c Mean±SD	7.9±2.5	8.3±2.3	p<0.001
Fpg Mean±SD	149±41.4	159.4±38.3	p<0.001

Table 4: Spearman's-Rho (ρ) coefficients correlation matrix between different biochemical parameters with statistical significance.

	HbA1c	FPG	CRP	Creatinine	Urea	Albuminuria
HbA1c	1					
FPG	0.76**	1				
CRP	0.26**	0.33**	1			
Creatinine	0.08	0.006	0.3**	1		
Urea	0.2*	0.19*	0.33**	0.62**	1	
Albuminuria	0.17*	0.24**	0.83**	0.343**	0.341**	1

** Correlation is significant at the 0.01 level (2-tailed), *Correlation is significant at the 0.05 level (2-tailed).

Spearman's-Rho (ρ) coefficients correlation matrix between different biochemical parameters with statistical significance

In the correlation analysis of different parameters, a strong and statistically significant correlation was found between CRP and albuminuria with a ρ value of 0.831 and a p value of 0.00. HbA1c shows a strong and statistically significant positive correlation with serum FPG levels, p=0.767 with a p value of 0.00. Creatinine shows a moderate and statistically significant positive correlation with serum level of urea p=0.624 with a p value of 0.00. All the other correlations between other

parameters were weakly correlated or negligibly correlated (Table 4).

Receiver-operating characteristic (ROC) analysis of CRP as a predictor of albuminuria

The area of 0.957 under the curve shows a very high predictive value of CRP for diagnosing significant albuminuria in a patient of T2DM. For a cut-off value of 15.5 mg/l of CRP, the sensitivity and specificity stand at 0.96 and 0.82 respectively for predicting albuminuria in patients of T2DM (Figure 1).

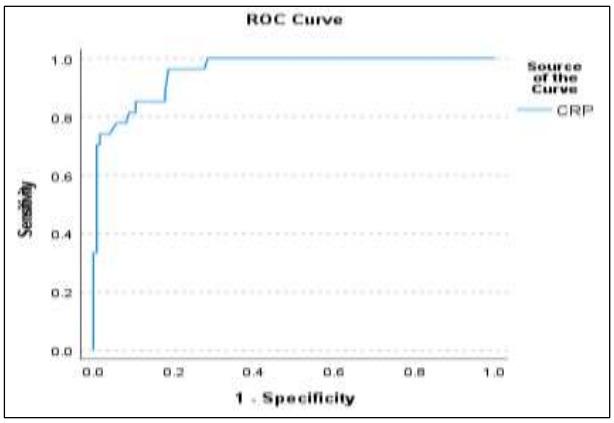

Figure 1: Receiver-operating characteristic (ROC) analysis of CRP as a predictor of albuminuria.

DISCUSSION

An expanding global health problem, type 2 diabetes mellitus (T2DM) is a disease caused due to insulin resistance and resulting in impaired glucose tolerance or hyperglycaemia.[17] Several pathophysiological disturbances contribute to impaired glucose tolerance in T2DM, such as obesity, an unhealthy diet, and physical inactivity.[18] Individuals with T2DM are at a significant risk of acquiring cardiovascular comorbidities as well as microvascular consequences such as nephropathy, and retinopathy.[19] The present study was conducted on patients diagnosed with T2DM to investigate the association of albuminuria with CRP and glycemic control. This study included male and female individuals diagnosed with T2DM under the age group of 30-55 years. Among these 28% belonged to the age group 30-44 years and 72% belonged to the age group of 45-55 years. The group with ages between 45-55 has a higher representation in the study group showing that they have a higher risk of developing T2DM. This finding is supported by other studies such as that of Hamid Z (2000) that showed an increase in the risk of developing impaired glucose tolerance and T2DM in people older than forty years.[20] Our study was a cross-sectional study on 150 individuals, 79 (53%) females and 71 (47%) males. Our study has shown higher prevalence of T2DM in females compared to males. This concurs with the findings of a study conducted by Al-Baghli et al.[21] But this in contradiction to the study by Jain et al, which found a similar rate of prevalence in both sexes or with the study of Sujata and Raman Thakur that's reported a higher prevalence of T2DM among men in India as compared to women.[22,23] The mean value of CRP in our study subjects was 12.9 mg/l with a std deviation of 9.8 mg/l.[24] In 110 (73.3%) subjects, who showed a CRP value higher than the higher limit of reference range, the mean value of CRP was 16.4 mg/l with standard deviation 9.3 mg/l, while in 40 (26.7%) subjects, who showed a CRP value with in the reference range, the mean value of CRP 3.3 mg/l with std deviation 1.6 mg/l. The difference between groups was statistically significant. ($p<0.001$).[25] The mean urea level in the Group with normal CRP levels was 30 mg/dl with std deviation of 8.5 mg/dl The mean urea level in the group with elevated CRP was 34.2 mg/dl with a std deviation of 13.5 mg/dl. The difference between the two groups was statistically significant ($p<0.001$). The mean creatinine level in the Group with normal CRP levels was 0.7 mg/dl with std deviation of 0.2 mg/dl. The mean creatinine level in the group with elevated CRP was 0.9 mg/dl with a std deviation of 0.3 mg/dl. The difference between the two groups was statistically significant ($p<0.001$). These above results suggest that elevated CRP levels are related to renal dysfunction at the level of glomerular endothelium. This finding is in agreement with the findings of Dabla PK.[26] The levels of HbA1c and FPG in normal CRP group mean values respectively 7.9% (±2.5%) and 149 mg/dl (±41mg/dl), while in increased CRP group mean values of HbA1c and FPG 8.3% (±2.3%) and 159.4 mg/dl (±38.3mg/dl), respectively. The difference between the groups was statistically significant. These above results suggest that elevated CRP levels are related to worsening of glycemic control. These findings are in agreement with the findings of Petchiappan et al.[27] The correlation between CRP and albuminuria was a statistically positive strong correlation with a ρ value of 0.831 and a p value of 0.00. This is in line with studies Kahraman C and Navarro JF.[28,29] This suggests that CRP may be directly involved in the pathological process of diabetic nephropathy. In the ROC analysis of CRP for its ability to predict albuminuria we found a strong predictive relationship as suggested by an area under the curve of 0.957. For a cut-off value of 15.5 mg/L of CRP in serum, the predictive sensitivity and specificity for albuminuria in T2DM were 0.96 and 0.82, respectively. This suggests that CRP can be used as an alternative screening method for the detection of albuminuria associated with early diabetic nephropathy.[30]

The major limitation of the study was that we have used spot morning sample to assess albuminuria in the study subjects. Using a 24-hour urine sample would have made our diagnosis of albuminuria more robust. The spot morning sample was used as the design of the study was a cross sectional study as there was a great risk of losing patients to follow up due to the cumbersome procedure of collecting and transporting a 24-hour sample to the lab.

CONCLUSION

From our results, we have been able to establish a strong predictive relationship between the level of CRP and albuminuria in T2DM. This strong correlation strengthens the hypothesis that inflammatory changes in T2DM are the primary drivers of the pathological processes in the complications of T2DM. It also suggests that inflammation is playing an important role in diabetic nephropathy, most probably employing inflammation-driven damage to the glomerular basement membrane. Our findings also have significant clinical and diagnostic suggestions. Measurement of albuminuria is a cumbersome process with the patient needing to collect a 24h urine sample and then transporting that nearly 2-litre sample to the lab. This is one reason why a number of our patients are lost to follow-up when test for albuminuria is suggested. Spot urinary creatinine and albumin ratio has been used as a method to overcome this problem. Now CRP levels, with a cut-off of 15.5 mg/l in serum with a sensitivity and specificity of 0.96 and 0.82, respectively, we have an effective screening tool for albuminuria using a single blood sample. Hence our study not only advances our understanding of pathogenesis of nephropathy in T2DM but also has the potential to add a new screening tool for early diabetic nephropathy.

ACKNOWLEDGEMENTS

Authors would like to thank all of the teaching members and technical employees at the biochemistry department and laboratory for assisting with the research work.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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Cite this article as: Rastogi A, Kumar A, Rastogi K, Kapoor S, Mahmood T. Study of association between C-reactive protein and albuminuria in type 2 diabetes mellitus. Int J Res Med Sci 2023;11:4136-41.