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Effect of glycemic control on serum C - reactive protein and development of nephropathy in type 2 diabetes mellitus.

Biochemistry

KEYWORDS: C - Reactive Protein; Diabetes Mellitus, Type 2; Glycemic control; HbA1C Hemoglobin A, Glycosylated.

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ABSTRACT

Aim: To assess the association between serum CRP concentration and the risk of development of diabetic nephropathy with glycemic control.

Material and Methods: A hospital based observational study was conducted on ninety clinically established non-insulin dependent diabetes mellitus (NIDDM) subjects having relevant clinical and laboratory measurements. Subjects were divided into two groups on the basis of urinary albumin excretion. Urinary albumin excretion was measured by qualitative Dipstick method. CRP and HbA_{1c} were measured using Immunoturbidimetric assay & Latex agglutination inhibition assay respectively.

Results: The mean HbA_{1c} level and serum CRP levels in diabetic subjects with microalbuminuria were higher and statistically significant ($P \leq 0.001$). The serum CRP levels and HbA_{1c} levels in NIDDM subjects were statistically significant and positively correlated ($P < 0.001$ and $r = 0.92$).

Conclusion: In this study, the serum CRP concentrations increased with increasing HbA_{1c} levels.which, suggests an association between glycemic control and systemic inflammation in people with established diabetes.

Introduction

Diabetes Mellitus is a metabolic disorder which is characterized by hyperglycemia and insufficiency of the secretion or the action of endogenous insulin. Although the aetiology of the disease has not been well defined, viral infections, autoimmune diseases, and environmental factors have been implicated [1].

Diabetic nephropathy is the leading cause of chronic kidney disease in patients starting renal replacement therapy [2] and is associated with increased cardiovascular mortality[3]. Diabetic nephropathy has been classically defined by the presence of proteinuria > 0.5 g/24 h. This stage has been referred to as overt nephropathy, clinical nephropathy, proteinuria or macroalbuminuria. In the early 1980s, seminal studies from Europe revealed that small amounts of albumin in the urine, not usually detected by conventional methods, were predictive of the later development of proteinuria in type 1 [4, 5, 6] and type 2 [4] diabetic patients. This stage of renal involvement was termed microalbuminuria or incipient nephropathy.

C-reactive protein (CRP), which is produced by the macrophages in the liver and adipocytes and is integrated in the acute-phase response pathways, is a very sensitive and well-characterized marker of subclinical low-grade inflammation [7]. Microalbuminuria is also associated with endothelial damage [8]. It can be anticipated that albuminuria level can be associated with higher levels of serum CRP [9] and activation of inflammatory pathways in progression of renal and cardiovascular atherosclerotic diseases can reflect in the CRP level.

Glycosylated hemoglobin (HbA_{1c}) reflects the average plasma glucose over the previous 8-12 weeks [10]. More recently there has been substantial interest in using it as a diagnostic test for diabetes and as a screening test for persons at high risk of diabetes [11]. HbA_{1c} could be used as a measure of glycemic control.

The present study was designed to evaluate the status of albuminuria and further, to determine the association between serum CRP and HbA_{1c} in type-2 diabetic patients. Regular screening of serum CRP and HbA_{1c} along with microalbuminuria as early markers of diabetic complications will be helpful in risk prediction of diabetic nephropathy and early intervention to ward off the complications.

Materials and methods

The present study was conducted from November 2015 to December 2016, in the Department of Biochemistry of S.M.S. Medical College and Hospital, Jaipur. All the 90 subjects diagnosed with NIDDM were taken from OPD/IPD of Department of Endocrinology & Department of Medicine of the hospital. The subjects having diabetes other than type 2 diabetes; known non-diabetic kidney disease, obstructive uropathy, urinary tract infection, fever, acute illness, congestive heart failure, malignancy and pregnancy; using uric acid lowering agents or diuretics were excluded.

The venous blood samples from the antecubital vein of NIDDM patients were taken after an overnight fasting for the estimation of serum CRP, blood sugar and for the estimation of HbA_{1c} & early morning midstream urine samples were collected in a sterile container for the estimation of albuminuria.

All subjects were divided into two groups on the basis of HbA_{1c}. Group 1 included controlled diabetic subjects with HbA_{1c} levels in between 6%-8%; Group 2 included uncontrolled diabetic subjects with HbA_{1c} levels more than 8%. Another grouping of NIDDM patients was done on the basis of urinary albumin excretion. Group - 1 included subjects with normoalbuminuria (urinary albumin excretion < 30 mg/day). Group - 2 included subjects with microalbuminuria (urinary albumin excretion of 30-300 mg/day).

Serum CRP was estimated by Immunoturbidimetric method (Meril, India) on semi auto analyser (Stat Fax 3300, USA). Estimation of serum glucose was done by Glucose Oxidase – Peroxidase method (Maxchem, India); & HbA_{1c} (%) was estimated by Latex agglutination inhibition assay (Randox, United Kingdom) on fully auto analyser (Randox Imola, United Kingdom). Urinary albumin excretion was estimated by semi-quantitative dipstick method (Clinitek Microalbumin 2 Reagent Strips) on Clinitek status system (Bayer Diagnostics, United Kingdom).

The data in the study was expressed as means ± SD. The statistical analysis was performed in Microsoft excel 2010 using unpaired t-test and Pearson's correlation coefficient. $P < 0.05$ was considered statistically significant.

Results and discussion

C-reactive protein (CRP) is an acute phase reactant and a sensitive marker of inflammation. Hyperglycemia can potentially promote the production of CRP. The present study showed a significant increase of CRP in subjects with diabetes, those who had HbA_{1C} level more than 8.0% and FBG 225.54 ± 58.43 mg/dl ($p < 0.001$) [Table no. 1]. This result was in agreement with findings previously reported by Abdrabo AA (2012) [12] and Sah SK et al. (2016) [13]. They observed that median hs-CRP level increases significantly with increasing number of metabolic syndrome components. In a study, median levels of hs – CRP were considerably higher in individuals with metabolic syndrome [14]. In a study by Engelsen et al. (2012), median hs – CRP levels were significantly higher in individuals with central obesity without the metabolic syndrome [15]. Wu et al. (2002) found an association of CRP with fasting glucose only among women [16]. However, Abdrabo AA (2012) observed that hs- CRP was significantly higher in all patients with high blood sugar regardless of their gender and CRP was higher in females than males. Kawamoto et al. (2011) reported that in women, only CRP increased significantly and progressively with increasing FBG ($r = 0.169$; $p < 0.001$) [17]. Recent clinical studies found that CRP is associated with diabetic nephropathy in both type 1 and type2 diabetes [18, 19, 20]. Tsioufis C et al. (2006) reported that elevated serum CRP level increased diabetic risk up to 2.7 times [21].

Hyperglycemia is known to stimulate the release of the inflammatory cytokines TNF-α and IL-6 from various cell types, and hyperglycemia can result in the induction and secretion of acute phase reactants by the liver in response to factors released by fat cells (adipocytes) [22, 23]. Due to that, elevated fasting blood glucose (FBG) is associated with elevated concentration of CRP [24].

In the present study, the mean HbA_{1C} level in diabetic subjects with microalbuminuria was higher and statistically significant ($P < 0.001$) (Table 2). Our study is in agreement with Gupta et al. (1991), who reported HbA_{1C} to be associated with microalbuminuria [25]. Levin SR et al. (2000) found that HbA_{1C} levels were significantly higher in diabetic subjects with microalbuminuria [26]. Hsu CC et al. (2012) concluded that In addition to mean HbA_{1C} values, HbA_{1C} variability, even measured as early as 2 years, is independently associated with the development of microalbuminuria in patients with type 2 diabetes. Baseline HbA_{1C} levels in subjects with normoalbuminuria and microalbuminuria were 8.0 ± 1.7% and 8.4 ± 2.0% respectively [27]. The association of glycemic control with microalbuminuria has been also well established by various authors [28,29,30,31].

The mechanism, that how the CRP is interrelated with albuminuria is not clear. It is known that polymorphism in the CRP gene is associated with serum CRP levels. The mean serum CRP level in diabetic subjects with microalbuminuria was higher and statistically significant ($P = 0.001$) [Table no.2]. Evidences supporting the pathogenic importance of CRP in diabetic nephropathy are coming out. Liu et al. (2011) found that CRP transgenic diabetic mice developed much more severe kidney injury than wild-type diabetic mice, as indicated by a significant increase in urinary albumin excretion and kidney injury molecule-1 abundance, enhanced infiltration of macrophages and T cells, and up regulation of pro-inflammatory cytokines and extracellular matrix. They also demonstrated that enhanced activation of TGF-β/SMAD and nuclear factor κB signalling pathways may be the mechanisms by which CRP promotes renal inflammation and fibrosis under diabetic conditions [32]. Ling Y et al. (2013) reported that elevated serum CRP levels were associated with albuminuria in Chinese type 2 diabetic patients. In their study, the mean values of CRP were 1.20 mg/L in subjects with normoalbuminuria and 2.30 mg/L in subjects with microalbuminuria ($p < 0.001$). Levels of serum CRP were significantly higher in diabetic subjects with microalbuminuria [33]. Our finding is in line with the previously reported associations of CRP with albuminuria in diabetic patients [18,19,20].

Further, we observed that serum CRP is positively correlated with the HbA_{1C} level ($r = 0.92$) and was statistically significant ($p < 0.001$) (Figure). It has been shown in different studies that HbA_{1C} correlates positively with CRP levels [34]. In a study from Turkey, they have found a positive correlation of hs-CRP with blood HbA_{1C}, fasting insulin and HOMA-IR [34]. King DE et al. (2003) have also shown that with increasing HbA_{1C}, the CRP levels tend to be higher [35]. In their statistical model, $HbA_{1C} > 9\%$ was a predictor of high CRP. However, a study from Iran has shown that CRP levels may be high in diabetes, even with low HbA_{1C} [36]. Laily Najafi et al. (2016) observed a significant association between CRP levels and HbA_{1C} levels at baseline [37]. These results imply a significant relation between inflammation and glycemic control in people with established diabetes [35]. It has been shown in different studies that HbA_{1C} correlates positively with CRP levels. Bandyopadhyay et al. (2013) reported that CRP and HbA_{1C} were statistically significant and positively correlated ($r = 0.79$; $p < 0.05$) [38].

Conclusion

In conclusion serum CRP levels are significantly higher in type 2 diabetic subjects with microalbuminuria and may contribute to the development of diabetic nephropathy. The present study demonstrated that HbA_{1C} is significantly associated with serum CRP. Glycemic control seems to affect the serum CRP levels in such patients. Assessment of these risk markers can, therefore, be helpful in early identification of patients at risk of developing such complications.

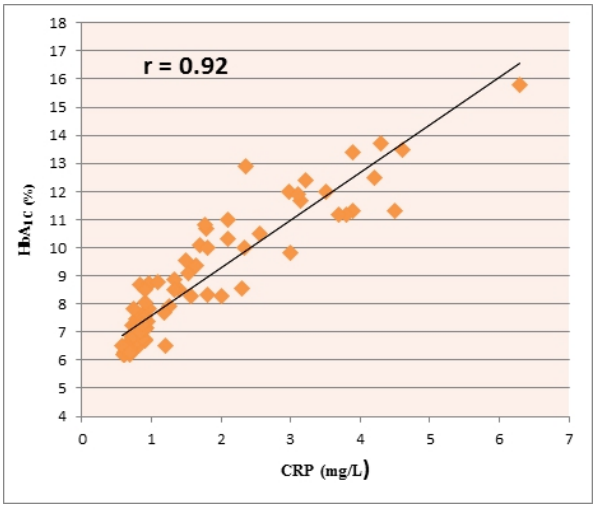
Table 1: Comparison of various biochemical parameters in controlled (n = 49) and uncontrolled (n = 41) diabetic subjects on the basis of HbA_{1C} levels.

Parameters	Controlled (6-8%)	Uncontrolled (>8%)	P-Value
Fasting blood Glucose (mg/dl)	150.89 ± 27.47	225.54 ± 58.43	< 0.001
CRP (mg/L)	0.79 ± 0.14	2.42 ± 1.27	< 0.001

Table 2 : Biochemical characteristics of study subjects

Variables	Type -2 Diabetes Mellitus		P value
	Normoalbuminuria n=56 (mean ± SD)	Microalbuminuria n=34 (mean ± SD)	
HbA_{1C} (%)	7.64 ± 1.60	9.92 ± 2.20	<0.001
Serum CRP (mg/L)	1.20 ± 0.86	2.10 ± 1.42	<0.001

Figure : Correlation between glycemic control (HbA1C) and C-Reactive protein



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