Correlation of hs-CRP with environmental risk factors of nephropathy in type 2 diabetes

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ABSTRACT

The objective of the present study was to investigate the association of hs-CRP levels with environmental risk factors of diabetic nephropathy like smoking, drinking alcohol, diet, age of diabetic patient, duration of diabetes, medication of diabetes, and blood pressure medication. A hospital-based quantitative study was conducted at the Department of Clinical Biochemistry of Manipal Teaching Hospital (MTH) Pokhara, Nepal, with 89 patients suffering from type 2 diabetes. Blood samples (n=89) from the patients were collected and the serums were separated. On the other hand, data on environmental risk factors of nephropathy were collected by using standard questionnaire. In this study, serum hs-CRP level was not found to be correlated with smoking (p=0.111), alcohol consumption (p=0.722), diet (p=0.496), duration of diabetes (p=0.519), age of diabetic patient (p=0.369), medication of diabetes (p=0.734), and blood pressure medication (p=0.625). Hence, our study concludes that serum hs-CRP value in type 2 diabetic patients is insignificantly correlated with the risk factors especially smoking, drinking alcohol, diet, duration of diabetes, age of diabetic patient, medication of diabetes, and medication of blood pressure.

Keywords

ARTICLE HISTORY

INTRODUCTION

Diabetic nephropathy, a multistage disorder, is a leading cause of End Stage Renal Disease (ESRD) (Agrawal and Dash, 2000). It affects 20-40% of diabetic patients, and is associated with enormous morbidity and mortality with greater health care cost (Crock, 2004). The development of nephropathy is rare in diabetic patients of less than 10-year duration (Martha and Femando, 1999). There is marked racial, ethnic and international disparity in the epidemiology of diabetic nephropathy. Native Americans, Hispanics (especially Mexican Americans) and African Americans have a much higher risk of developing ESRD as compared to non-Hispanic whites with type 2 diabetes (Venugopal et al., 2002; Jane, 2003).

C-Reactive Protein (CRP) is an acute phase protein produced exclusively in the liver due to several factors that are released from fat cells and macrophages. It is the member of the pentaxin family proteins (Pepys, 2003). The CRP was considered as the first pattern recognition receptor (PRR) to be cared (Erlinger et al., 2004).

The CRP can be expressed in several conditions, for example, bacterial, fungal, and viral infections; malignancy; tissue injury or necrosis; and rheumatic and other inflammatory diseases. These conditions cause release of interleukin-6 (IL-6) and other cytokines triggering to synthesize CRP and fibrinogen in liver (Pepys, 2003). During the acute phase response, its levels rapidly increase within two hours of acute insult and reaches to a peak within 48 h. With resolution of the acute phase response, the CRP level declines within 18 h. As the physiological role, CRP activates the

complement system through binding to phosphocholine that is expressed on the surface of dead or dying cells (including some bacteria). Thus, it causes the clearance of necrotic and apoptotic cells. The CRP also takes part in innate immunity as an early defense system against infections (Liu et al., 2011; Baluch et al., 2011).

Therefore, to prevent the early incidence of kidney diseases and other drastic consequences, an updated technique is needed, that will be helpful to the people to know their health status. Hence, the present study aimed at studying the association of environmental risk factors with the level of serum hs-CRP.

MATERIALS AND METHODS

This is an analytical, quantitative, hospital-based study conducted at the Department of Clinical Biochemistry of Manipal Teaching Hospital (MTH) Pokhara Nepal to predict the association of hs-CRP with the environmental risk factors of diabetic nephropathy in type 2 diabetes. A total of 89 patients aging more than 30 years who were suffering from type 2 diabetes, and visited the outpatients and inpatient department of MTH for their routine medical checkup, were included in the study.

Questionnaire and Data Collection: The study data were collected by personal interview using a pretested questionnaire containing demographic condition, past medical history, family history, duration of diabetes, history of treatment of diabetes mellitus (DM) and hypertension, smoking, alcohol behavior, and dietary habit. The subjects were also asked for the presence of any other complication of DM.

Sample Collection: Overnight fasting diabetic patients were selected for blood collection and about 3 mL venous blood was collected from each patient, of which 2 mL of whole blood from each was added in fluoride vial for glucose measurement and remaining blood was added in plain test tube for serum hs-CRP measurement. The blood collected in the tubes was allowed to clot at room temperature, and then centrifuged at 3000 rpm for 10 min. Blood glucose was analyzed in the same day. And, the remaining serum was preserved in eppendroff tube at -20°C for hs-CRP estimation.

Biochemical analysis: Plasma glucose level was measured by enzymatic end point glucose oxidase/peroxidase method (Nan, 2010). And serum hs-CRP was measured by sandwitch ELISA method (Chen-Chun et al., 2003).

Data Analysis: The collected data were entered in Microsoft Excel, and checked for any inconsistency. The Pearson correlation coefficient was used to find out the correlation between various environmental factors with hs-CRP. The value of $p<0.05$ was taken as significant. All the analyses were carried out by using SPSS 15.1 version.

RESULTS AND DISCUSSION

The present study was conducted on 89 type 2 diabetic patients. Among them 51 were male and 38 were female patients aging between 30 and 70 years. Overall, serum hs-CRP were not correlated with smoking ($p=0.111$), alcohol consumption ($p=0.722$), diet ($p=0.496$), duration of diabetes ($p=0.519$), age of diabetic patients ($p=0.369$), and medication of diabetes ($p=0.734$). In the same way, serum hs-CRP was not also significantly correlated with blood pressure medication ($p=0.625$).

Among the 89 samples, 60 had high fasting plasma glucose level. On the other hand, 22 patients were at high risk of diabetic nephropathy (hs-CRP value above 6 mg/L), 44 patients were at low risk (hs-CRP value less than 2 mg/L), and 23 patients were at moderate risk (serum hs-CRP value between 2-6 mg/L) (**Table 1**).

Table 2 shows the association of serum hs-CRP with the risk factors of nephropathy. No significant association was found between serum hs-CRP level and the environment risk factors (**Table 2**).

Several recent studies have shown that the patients suffering from type 2 DM and nephropathy exhibit high levels of diverse acute-phase markers of inflammation including CRP, serum amyloid A, fibrinogen and IL-6 (Chen-Chun et al., 2003; Crook, 2004; Mittal et al., 2010; Khalaf, 2010; Jiji, 2013). One study has reported that 96.2% of the type 2 diabetic nephropathy patients have raised CRP levels (Khalaf, 2010). These evidences prove that the subclinical chronic inflammation is involved in the pathogenesis of diabetic nephropathy (Choudhary, 2002). Similar finding of hs-CRP level was recorded in our study.

Hyperglycemia is a precondition for developing glomerular basement membrane (GBM) thickening and mesangial expansion, which may not present during early stage of diabetes but can be diagnosed 2 to 5

Table 1. Biochemical characteristics of the risk factors of diabetic nephropathy.

Parameter	Low	Normal	High	Total	Normal Range
Fasting Plasma Glucose	0	29	60	89	70-110mg/dL
Serum hs-CRP	44	23	22	89	Low risk =<2mg/L
	(low risk)	(moderate risk)	(high risk)		Moderate risk =2-6 mg/L
					High risk =>6 mg/L

Table 2. Association of serum hs-CRP with the environmental risk factors of nephropathy.

Variables	Concentration of serum hs-CRP			χ^2 value	*p*-value
	High risk	Low risk	moderate		
Smoking					
Smoker	6(46.2%)	6(46.2%)	1(7.7%)	7.524	0.111
Non smoker	14(20.6%)	36(52.9%)	18(26.5%)		
Previous smoker	2(25.0%)	2(25.0%)	4(50.0%)		
Drinking Alcohol					
Alcoholic	3(33.3%)	3(33.3%)	3(33.3%)	2.075	0.722
Non alcoholic	15(23.1%)	35(53.8%)	15(23.1%)		
Previous alcoholic	4(26.7%)	6(40%)	5(33.3%)		
Diet					
Vegetarian	1(11.1%)	6(66.7%)	2(22.2%)	1.402	0.496
Non vegetarian	21(26.3%)	38(47.5%)	21(26.3%)		
Duration of diabetes					
1 year duration	2(33.3%)	1(16.7%)	3(50%)	3.328	0.519
Less than 10 yrs	16(23.2%)	36(52.2%)	17(24.6%)		
Greater than 10yrs	4(28.6%)	7(50.0%)	3(21.4%)		
Age of patients					
35 to 50 yrs	4(21.1%)	12(63.2%)	3(15.8%)	1.996	0.369
Above 50 yrs	18(25.7%)	32(45.7%)	20(28.6%)		
Medication of diabetes					
Oral medication	18(24.0%)	38(50.7%)	19(25.3%)	2.008	0.734
Receiving insulin	1(50.0%)	0(0.0%)	1(50%)		
No medicine	3(25%)	6(50.0%)	3(25%)		
Pressure medication					
Yes	14(25.9%)	28(51.9%)	12(22.2%)	0.939	0.625
No	8(22.9%)	16(45.7%)	11(31.4%)		

years after onset of hyperglycemia. In chronic hyperglycemia, non-enzymatic glycation of amino acids, lipids and lipoproteins may occur. The formation of advanced glycation end-products (AGEs) has long been recognized as a fundamental mechanism of cellular injury in diabetes. The accumulation of AGEs accelerates atherogenesis, basement membrane thickening, increased extracellular matrix, and mesangial fibrosis. This process leads to eventual glomerulosclerosis and renal failure (Martha and Femando, 1999; Pasceri, 2000; Venugopal et al., 2002; Crook, 2004).

Our study was revealed insignificant association of smoking, age of diabetic patients, diet, medication of diabetes, and alcoholic habit of the patients with serum hs-CRP level in type 2 diabetic patients; however, Chen-Chung et al. (2003) showed that there was significant association between them. In the same way, our research revealed that there was no significant association between blood pressure medication with the risk of development of nephropathy, as described

by Chen-Chung et al. (2003) showed the similar result. We also found that there was insignificant association between serum hs-CRP levels and blood pressure medication.

CONCLUSION

The present investigation demonstrates that serum hs-CRP level is insignificantly correlated with diet, alcohol consumption, duration of diabetes, age of patients, and medication of blood pressure, and diabetes in type 2 diabetes. It indicates that the elevation in serum hs-CRP value increases the risk of diabetic nephropathy, but increase in hs-CRP level is independent of its environmental risk factors.

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