Original Article

Assessment of hs-CRP with Serum Urea in Type-2 Diabetic Patients in Pokhara, Nepal

Jay Prakash Sah*[1], Chandra Kant Yadav[1] and Dipendra Kumar yadav[2]

[1]Department of Medical Laboratory Science, School of Health and Allied Sciences, Pokhara University, Lekhnath -12 Kaski, Nepal
[2]Department of Public Health, School of Health and Allied Sciences, Pokhara University, Lekhnath -12 Kaski, Nepal

Address for Correspondence

Department of Medical Laboratory Science, School of Health and Allied Sciences, Pokhara University, Lekhnath -12 Kaski, Nepal.
E-mail: shahjayprakash1@gmail.com

ABSTRACT

High-sensitivity C-reactive protein (hs-CRP) is an acute phase protein produced as a marker of inflammation. It may play role as predictor of inflammation in patients with type 2 diabetes. The aim of this study was to estimate blood hs-CRP and urea level and also to determine association between them.
The relationship between inflammation and blood urea level in type-2 diabetes mellitus has not yet been reported in Nepalese population. So a quantitative laboratory based, descriptive, analytical study were done by enrolling 89 type 2 diabetic patients conducted at Tertiary Care Hospital. We analyzed serum concentrations of high sensitive C-reactive protein (hs-CRP), urea, blood glucose and family history of the patients. In our study, we found the significant association between serum hs-CRP and Serum Urea level (P values<0.01).We also found a significant association between serum hs-CRP and Blood Glucose level (P values=0.01). Furthermore, serum hs-CRP was not correlated with both family history of patients (P >0.197) and sex(P>0.265).
This study concludes that the increase in serum hs-CRP value in type 2 diabetic patients increases the risk of diabetic nephropathy and thus increase the value of serum urea level. And there is no correlation of serum hs-CRP and urea level with the risk factors especially sex and family history of type 2 diabetes.
The significance of these findings is a new diagnostic tool for early screening of diabetic nephropathy in type 2 diabetic patients to prevent from further complication.

Keywords: Blood urea, High sensitivity C-reactive protein, Type 2 diabetes.

INTRODUCTION

Diabetes mellitus, is a clinical syndrome characterized by hyperglycemia, that mainly occurs due to impaired insulin secretion and its function.[1] Type 2 diabetes usually occurs in adult over 35 years old.[1,2] The risk factors for type 2 diabetes are obesity, poor diet, sedentary lifestyle, increased age, family history and metabolic syndrome.[1] Although diabetes mellitus is a metabolic disease, it may lead to coronary artery disease (CAD), peripheral vascular disease (PVD) and Cerebro-vascular disease (CVD).[5] Indeed CAD accounts for more than 50% of the mortality among type 2 diabetic subject.[14] There are a number of several metabolic defects like dyslipidemia, hyperuremia, hyperuricamia, hypertension which have been shown to predict complication in type 2 diabetes.[35] Excess cholesterol can be deposited in blood vessel walls that can lead to atherosclerosis and cardiovascular disease.[27, 31] In the same way, high blood urea indicate the abnormality of kidney function.[22] Factors such as age, sex and genetic factor influence serum urea level in diabetic patients. Certain aspects of the lifestyle including diet, physical activity, diabetes control level, alcohol consumption, smoking status also affect the serum urea in diabetic patients.[4,14,19] Abnormal blood urea is the symptom of Nephropathy. Therefore, there is a need for early detection of Nephropathy.[4]

CRP is an acute-phase reactant belonging to the pentraxin family. It is almost exclusively produced in the hepatocytes under the control of cytokines.[16] The major functions of CRP include binding to various ligands on damaged tissue followed by propagation of both anti- and proinflammatory effects.[6-12] Recent evidence indicates that CRP plays an active role in atherosclerosis[27].

Hyperglycemia is an associated factor for increment of serum CRP levels, in uncontrolled type-2 diabetic subjects.[7] In one study, it was reported that elevated serum high sensitive C-reactive protein (hs-CRP) level increased diabetic risk up to 2.7 times. And it is an earlier inflammatory biomarker which has been reported to be associated with diabetes, CAD, nephropathy and End stage renal disease.[8]

Therefore, it can be anticipated that serum urea level can be associated with higher levels of serum CRP and activation of inflammatory pathways in progression of renal and cardiovascular atherosclerotic diseases. Accordingly, we carried out a study on patients with type 2 DM to investigate the relationship between serum hs-CRP and serum urea level as a marker of kidney damage.

MATERIALS AND METHODS

Study design

The study design was descriptive cross sectional hospital based study. A total of 89 type- 2 diabetic patients attending the outpatient and inpatient department of Manipal Teaching Hospital, Pokhara, Nepal have been screened for serum urea, hs-CRP after obtaining informed consent. Detailed interview by using a structured questionnaire was also documented. The study was approved by the Institutional ethical committee on human research. Patients with Type I Diabetic Patients, Patients <30 years, Gestational Diabetes, Muscular Dystrophy, Dehydration, Pyelonephritis, Urinary tract obstruction, the diseases due to other than diabetes were excluded from the study.

Blood collection & Biochemical assay

Blood samples were taken in the morning after 12 to 14 hours overnight fasting subjects. Blood sample were collected in sterile tubes, centrifuged at 1500

rpm for 10 minutes and serum was stored at 4°C or further stored at -80°C until assayed. Fasting Blood Glucose (FBG) and Postprandial (PPBG) level were measured by an enzymatic GOD-POD method.[26] Serum Urea level was estimated by standard enzymatic urease method[22]. All chemicals and reagents of excellent quality obtained from Tulip Company Ltd, India. And marker of inflammation high sensitivity C-reactive protein (hs-CRP) was measured by solid phase Sandwich Enzyme Linked Immunosorbent Assay Method.[7]

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

RESULTS

A total of 89 patients were enrolled in the study. Among them 51 were male and 38 were female patients of the age ranging from greater than 30 to 72 years. Based on serum urea estimation, 80 diabetic patients had normal serum urea level and 9 patients had high urea level. Similarly, serum hs-CRP was significantly higher in all diabetic patients but nearly 50% diabetic patients were found to be at low risk when classified the patients on the basis of serum hs-CRP level as Low risk=<2mg/L, Moderate risk=2-6mg/L, High risk=>6 mg/L (This classification is on the basis of American Diabetic association).

Table 1, shows that out of 89 samples analyzed, 80 patients have normal urea level and 9 patients have high value. 29 have normal fasting plasma glucose level. On the other hand, 60 samples have high fasting plasma glucose level. Similarly, 22 patients are at the high risk of diabetic nephropathy (hs-CRP value above 6mg/L), 44 patients are at low risk (hs-CRP value less than 2mg/L) and 23 patients are at moderate risk (serum hs-CRP value between 2mg/L-6mg/L).

*2-tailed P value is significant at the 0.01 level

Table 2, shows the significant correlation between values serum hs-CRP with the fasting plasma glucose of the patients. P value between serum hs-CRP and serum urea was found to be significantly correlated ($\gamma=0.445$, $p< 0.01$). And serum hs-CRP and fasting plasma glucose were also significantly correlated ($\gamma= 0.258$, $p=0.01$).

Table 3, shows that there is no significant association of serum urea with the other risk factors like sex, family history of diabetic nephropathy.

Table 4, shows that there is no association of serum hs-CRP with the other risk factors like family history and sex of Diabetic nephropathy.

DISCUSSION

In several studies, it has been reported that there is a clear association of serum urea with high levels of acute-phase marker C-reactive protein (CRP) in diabetic patients[8,25]. CRP is an inflammatory marker reflected by high serum level in low grade inflammation. It is produced by the macrophages in the liver and adipocytes and is integrated in the acute-phase response pathways[20, 21]. CRP may play an important role in induction of serum urea level which is the indicative of diabetic nephropathy. So Serum urea estimation along with CRP is a strong predictor for development of clinical diabetic nephropathy and their early diagnosis may help to prevent the further progression of kidney disease. (See figure 1.)

Therefore, in order to support for the detection of diabetic nephropathy, we tried to find a relation between hs-CRP and serum Urea level.

Sugam S *et al* found that diabetic patients have high serum urea in compare to non-diabetic patients. They reported that 18 out of 103 diabetes samples have high urea level. An another study also showed that the increase in mean values of serum urea i.e.136.03 ± SD 74.6 mg/dl in kidney disease patients with Diabetes mellitus[23]. We also found the similar result.

In our study, we found the significant increase in serum hs-CRP level and strong association between serum hs-CRP and Serum Urea level. Our finding was also strongly correlated with the finding obtained by Salim Jasim Khalaf [26]. On the other hand, we found there is no significant association of family history and sex with serum hs-CRP level but some reports have shown a strong correlation among them[25]. In the same way we found insignificant correlation between family history and sex with blood urea level in diabetic patients.

CONCLUSION

This present descriptive, analytical, quantitative study carried out in 89 type 2 diabetic patients conducted at Manipal teaching Hospital, Pokhara Nepal has clearly shown that serum hs-CRP level is significantly correlated with serum urea level in type 2 diabetes. It indicates that the increase in serum hs-CRP value in type 2 diabetic patients increases the risk of diabetic nephropathy and thus increases the value of serum urea level. This study has also shown that serum hs-CRP and blood urea are not significantly correlated with risk factor like sex and family history of type 2 diabetes.

Therefore, these findings may influence policies to choose association of hs-CRP and serum urea level study for early screening of diabetic nephropathy in type 2 diabetic patients to prevent from further complications.

ACKNOWLEDGEMENT

We are grateful to the Director and entire team of Laboratory medicine of Manipal Teaching Hospital, Pokhara, Nepal, for the opportunity and the help rendered to carry out the investigation in their medical laboratory. Our appreciation also goes to Anjali Negi Sah, Ajita Shah and Adarsh Shah whose valuable contribution for enormous support for funding and searching article for report writing.

REFERENCES

1. Kasper DI BG, Fauci AS, Hauser SL, Longo DL, Jameson JL. *Harrison's Principles of Internal Medicine*. 3. 2005; 16(3).
2. Rabin R. Clinical Laboratory Medicine: Clinical Application of Laboratory Data. USA, Mosby. 1995(6):453-73.
3. Agarwal SK, Dash SC. Spectrum of renal diseases in Indian adults. *J Assoc Physicians India*. 2000; 48(6):594-600.
4. American Diabetes Association. Nephropathy in diabetes (Position Statement) *Diabetes Care*. 2004; 27(1):79-83.
5. Crook M. Type 2 diabetes mellitus: a disease of the innate immune system? An update. *Diabet Med*. 2004(21):203-7.
6. Dalla VM, Mussap M, Gallina P. Acute-phase markers of inflammation and glomerular structure in patients with type 2 diabetes. *J Am Soc Nephrol*. 2005(16):78-82.
7. Martha RM, Fernando GR. Increased levels of CRP in Non-controlled Type II diabetic subjects. *Journal of Diabetes and its complications*. 1999(13): 211-215.
8. Blake GJ RP. C-reactive protein and other inflammatory risk markers in acute coronary syndromes. *Journal of the American College of Cardiology*. 2003; 41(4):37-42.
9. Wang CH, Weisel RD, Fedak PW, Dumont AS, Szmitko P, Li RK, Mickle DA, Verma S. C-reactive protein upregulates angiotensin type 1 receptors in vascular smooth musles. *Circulation*. 2003(107):1783-90.
10. Pasceri V WJYE. Direct proinflammatory effect of C-reactive protein on human

endothelial cells. *Circulation*. 2000 (102):2165-8.
11. Venugopal SK, Yuhanna I, Shaul P. Demonstration that C reactive protein decreases eNOS expression and bioactivity in human aortic endothelial cells. *Circulation*. 2002(106):1439-41.
12. Ridker PM, Bassuk SS, Toth PP. C-reactive protein and risk of cardiovascular disease: evidence and clinical application. *Current Atherosclerosis Reports*. 2003; 5 (5): 341–49.
13. Bethesda, MD: National Institutes of Health, National Institute of Diabetes and and Kidney Diseases. 2004.
14. M B. Biochemistry and molecular cell biology of diabetic complications. *Nature*. 2001; 414:813-20.
15. M C. Pathogenesis, prevention and treatment of diabetic nephropathy. *Lancet*. 1998.
16. Pepys MB. C-reactive protein: a critical update. *J Clin Invest*. 2003; 111(12):1805-12.
17. Erlinger TP PE, Rifai N, Helzlsouer KJ. C-reactive protein and the risk of incident colorectal cancer. *JAMA*. 2004; 291(5):585-90.
18. Bhattarai MD SD. Learning the lessons – preventing type 2 diabetes in Nepal. *Diabetes voice*. 2007; 52(2):9-10.
19. Chowdhury TA LS. Complications and cardiovascular risk factors in South Asians and Europeans with early-onset type 2 diabetes. *QJM*. 2002; 95:241-6.
20. Urooj Taheed Baluch IGK, Niazi R, Nighat B, Tahir F. C- Reactive Protein As A Low Grade Inflammatory Marker in type 2 diabetic nephropathy. *Ann Pak Inst Med Sci*. 2011; 7(4):217-21.
21. Liu F CH, Huang XR, Chung AC, Zhou L, Fu P. C-reactive protein promotes diabetic kidney disease in a mouse model of type 1 diabetes. *Diabetologia*. 2011; 54:2713-23.
22. Sugam S, Shrestha R, Poudel B, Sigdel M. Serum Urea and Creatinine in Diabetic and non-diabetic Subjects. *Journal of Nepal Association for Medical Laboratory Sciences*. 2008; 9(1):11-2.
23. Mittal A SB, Kumar A, Chandrasekharan N, Sunka A. Diabetes mellitus as a Potential Risk Factor for Renal Disease among Nepalese. *Nepal journal of Epidemiology*. 2010; 1(1):22-5.
24. Jiji Inassi VR. Role of duration of diabetes in the development of nephropathy in type 2 diabetic patients. *National Journal of Medical Research*. 2013; 3(1):1-8.
25. Chen-Chung F Y-ML, Jer-Chuan Li1, Du-An Wu. Association of C- reactive Protein and Traditional Risk Factors with Nephropathy in the Elderly Patients with Diabetes. *Taiwan Geriatrics & Gerontology*. 3:202-10.
26. Khalaf SJ. Study of some biochemical markers in diabetic patients. *Tikrit Medical Journal*. 2010; 16(2):84-7.
27. Sun H, Koike T, Ichikawa T. C-reactive protein in atherosclerotic lesions: its origin and patho-physiological significance. *American Journal of Pathology*. 2005; 167(4): 1139–48
28. Sacks DB, Bruns DE, Goldstein DE, Maclaren NK. Guidelines and Recommendations for Laboratory Analysis in the Diagnosis and Management of Diabetes Mellitus. *Clin Chem*. 2002; 48(3):436-72.
29. Azza ME, Tarek MA, Rizk AE, Wafaa AM. Role of hypertension and metabolic abnormalities in the development of diabetic nephropathy among Egyptian patients with type 2 diabetes. *Nature and Science*. 2011; 9(7):220-8.
30. Lehto S, Ronnemaa T, Pyorala K, Laakso M. Cardiovascular risk factors in patients with type II diabetes. *Diabetologia*. 2000(43):148-55.
31. Leibson CL, Palumbo PJ, Weston SA and Killian JM. Coronary atherosclerosis in diabetes mellitus: *J. Am. Coll. Cardiol*. 2002(40):946–53.

Table 1. Serum urea, blood glucose and serum Hs CRP level in diabetic patients

Parameters	Patients having low value (%)	Patients having normal Values (%)	Patients having high Values (%)	Total Patients (%)	Normal Range
Serum Urea	0	89.88	10.11	100	15-45 mg/dl
Fasting Plasma Glucose	0	32.58	67.41	100	70-110mg/dl
Serum hs-CRP	49.3(Low risk)	25.84 (Moderate risk)	24.71 (High risk)	100	Low risk=<2mg/L Moderate risk 2-6mg/L High risk=>6 mg/L

Table 2. Correlations of serum hs-CRP level with serum urea, fasting plasma glucose level of diabetic patients

Parameters	Serum Urea level	Fasting plasma glucose
Serum hs-CRP of the patients (γ) Spearman's correlation coefficient	0.445	0.258
2 tailed P value	0.001**	0.014**
Total Number of sample	89	89

Table 3. Correlation of serum urea level with risk factors of diabetic nephropathy

Variables	High serum Urea (>45mg/dl)	Low Serum Urea level (<15mg/dl)	Normal Serum urea level (15-45mg/dl)	Chi Square test	P values
Family History					
Yes	7(14.3%)	1(2.0%)	41(83.7%)	1.025[a]	0.599
No	3(7.5%)	1(2.5%)	36(90.0%)		
Sex					
Male	8(15.7%)	0(0.0%)	43(84.3%)	4.857[a]	0.08
Female	2(53%)	2(53%)	34(89.5%)		

Table 4. Correlation of serum hs-CRP level with risk factors of diabetic nephropathy

Variables	Serum hs- CRP level (>6mg/l) High	Serum hs- CRP level (<0.5mg/l) Low	Serum hs- CRP level (<2mg/l) Normal	Chi Square test	P values
Family History					
Yes	14(28.6%)	20(40.8%)	15(30.6%)	3.254[a]	0.197
No	8(20%)	24(60%)	8(20.0%)		
Sex					
Male	11(21.6%)	29(56.9%)	11(21.6%)	2.656[a]	0.265
Female	11(28.9%)	15(39.5%)	12(31.6%)		

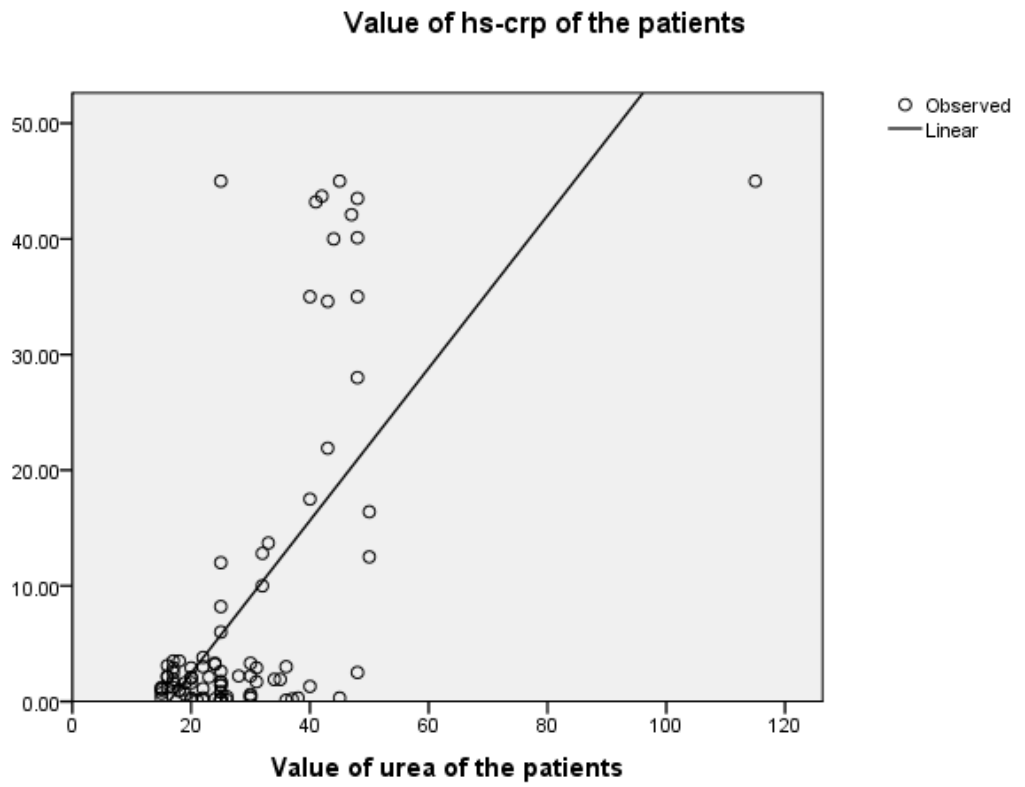

Figure 1. Correlation between hs-CRP and serum urea in type II diabetic patients (P<0.01, γ= 0.445)