Original Article

A Comparative Study of High Sensitivity C-Reactive Protein and Metabolic Variables in Type 2 Diabetes Mellitus with and without Nephropathy

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ABSTRACT

Introduction: Diabetic nephropathy is a serious chronic complication of Type 2 Diabetes Mellitus (T2DM) which impairs the quality of life, leading to increased morbidity and mortality. The high sensitivity C-reactive protein (hs-CRP) is an acute phase reactant which acts as a non-specific systemic marker of inflammation.

Aim: To find out the relationship between serum hs-CRP and metabolic variables in Type 2 diabetic patients with and without nephropathy.

Materials and Methods: The study group consists of 96 subjects that include non-diabetic healthy controls, Type 2 diabetic patients without any complications and patients with diabetic nephropathy. The study group was composed of both genders, aged 31-70 years, who reported after 10-12 hours overnight fasting; then, venous blood and fresh urine samples were collected, in the morning from all the study subjects. Data were analysed using Statistical Package for the Social Sciences (SPSS). Independent t-test was used to compare between the groups and Chi square test was used to find out the relationship between serum hs-CRP and metabolic variables.

Results: The results showed a significantly (p<0.05) increasing trend of serum hs-CRP with the degree of microalbumin excretion and the severity of nephropathy in Type 2 diabetic patients. The result showed a significant (p<0.05) relationship between hs-CRP and the metabolic variables like Fasting Blood Glucose (FBG), Post Prandial Blood Glucose (PPBG), Total Cholesterol (TC), Triglycerides (TG), LDL-Cholesterol (LDL-C), TC:HDL-Cholesterol (HDL-C) ratio and estimated Glomerular Filtration Rate (eGFR) and no significance (p>0.05) between hs-CRP and HDL-Cholesterol in both diabetic and diabetic nephropathy subjects.

Conclusion: Hs-CRP was strongly associated with the metabolic variables and predictors of cardiovascular risk in Type 2 diabetes mellitus with and without nephropathy. The hs-CRP might be considered as a predictor or illness indicator for the development of nephropathy and cardiovascular risk in Type 2 diabetic patients.

INTRODUCTION

Diabetes mellitus may be defined as a metabolic disorder characterized by hyperglycemia which leads to chronic complications such as diabetic nephropathy and cardiovascular diseases. These complications usually appear in the second decade of the hyperglycemia and the risk increases with the severity of hyperglycemia [1]. Incidence of Type 2 diabetes is enhancing globally and has reached wide ranging proportions in many countries. The recent evaluation by the International Diabetes Federation (IDF) revealed the number of people affected by the diabetes mellitus was 382 million which is predicted to increase to 592 million by 2035. IDF also estimated that about 8.3% of world population and 65.1 million of adult people in India are affected by diabetes [2]. A large body of data showed that the prevalence of Type 2 diabetes varies considerably between urban and rural populations and reveal the prevalence of diabetes has increased to 18.6% in urban and 9.2% in rural population in India with significant regional variations [3].

Diabetic nephropathy is one of the chronic complications of Type 2 diabetes mellitus which impair the quality of life leading to increased morbidity and mortality. Microalbuminuria is the continuous elevation of albumin excretion in urine in a range of 20 mg/L-200 mg/L in early morning urine [4,5]. Microalbumin is one of the independent risk factor for vascular diseases like cardiovascular disease and is integrated with endothelial dysfunction and chronic inflammation [6]. Innate immune system and low grade inflammation are significantly associated to the incidence and severity of Type 2 diabetes mellitus and its complications [7,8]. The hs-CRP is an acute phase reactant which acts as a non-specific systemic marker of inflammation. It is a pentameric, globular protein synthesized by liver and is considered as an effective marker for long term risk assessment [9-11]. Low grade systemic inflammation may be used to predict the onset of cardiovascular disease and Type 2 diabetes mellitus [12,13]. Obesity, hypertension and dyslipidemia along with altered level of lipoproteins are associated with glycation, oxidation and insulin resistance in Type 2 diabetes mellitus. Endothelial dysfunction contributes to the development of cardiovascular disease via vascular tone dysregulation, growth, thrombogenicity, and inflammation. Hemostatic and inflammatory biomarkers of endothelial dysfunction like CRP have been associated with cardiovascular disease [14,15].

The present study was carried out to find out the relationship between serum hs-CRP and metabolic variables in Type 2 diabetic patients with and without nephropathy. The study signifies an increasing trend of serum hs-CRP with the degree of microalbumin excretion and the severity of nephropathy in Type 2 diabetic patients. In addition, microalbumin is one of the risk factor for cardiovascular disease and might be linked with chronic inflammation which leads to the alteration in the serum hs-CRP levels. This study adds the importance of measuring hs-CRP as a routine test in Type 2 diabetic patients to reduce the subsequent risk of diabetic nephropathy.

MATERIALS AND METHODS

The current pilot, case-control study has been conducted in MES Medical College and Hospital, Kerala, India, during May 2009 to October 2009. Ethical approval was taken from Institutional Ethical

Committee and informed consent was obtained from all individual participants included in the study. The blood and urine samples were collected from 96 subjects which include both male and female. The study groups include 32 non-diabetic healthy controls, 32 Type 2 diabetic patients without any complications and 32 diabetic nephropathy patients. The sample size was considered as per convenient sampling and availability of data in the short duration of the study. Patients with Type 2 diabetes mellitus within age group of 31-70 years were reported after 10-12 hours overnight fast and 3 ml of venous blood sample from the study subjects were collected for the analysis. Then, 1 ml of blood sample was transferred to grey coloured blood collection vial that contain sodium fluoride-potassium oxalate mixture and was used for fasting plasma glucose estimation. In addition, 2 ml of blood sample was transferred to red coloured vial that contain clot activator and was used for serum determination. The subjects reported again after two hours of heavy breakfast and 1 ml of blood was collected in grey vial for post prandial blood glucose estimation. The blood samples were centrifuged at 2000 rpm for the separation of plasma or serum and transferred to microcentrifuge tubes for further analysis.

Inclusion criteria: Type 2 diabetic patients without any complications, Type 2 diabetic patients with nephropathy and non-diabetic healthy controls.

Exclusion criteria: Type 1 diabetic patients, gestational diabetes mellitus, diabetic ketoacidosis, smokers, alcoholics, chronic illness and patients on treatment with lipid lowering agents, anti-inflammatory drugs, multivitamin and aspirin were excluded from the study.

BMI was calculated by dividing weight (in kg) with height (in m^2). SBP was measured by Sphygmomanometer. Waist and hip circumferences were measured to calculate Waist/hip ratio. The separated samples were analysed for FBG, PPBG, TC, TG, HDL-C, LDL-C and creatinine on fully automated analyser (VITROS 250 Chemistry Analyser, Johnson and Johnson Company) with the reagents supplied by Johnson and Johnson Company. VITROS 250 Chemistry System is based on the Dry slide Technology. The thin film dry slide is the key element of VITROS technology.

Serum hs-CRP was measured by Quantia-CRP-US turbidimetric immunoassay kit supplied by Coral Clinical Systems. Quantia-CRP-US assay kit is based on the principle of agglutination reaction. The test specimen is mixed with Quantia-CRP US® latex reagent, then activation buffer was allowed to react. Presence of CRP in the test specimen results in the formation of an insoluble complex producing a turbidity, which is measured at 540 nm. The fresh, early morning urine samples collected from the study subjects were used for microalbumin estimation by a solid phase, sandwich-format quantitative immunometric assay using NycoCard® READER II. When the diluted urine flows through the membrane coated, immobilized antibodies capture the albumin molecules. Albumin trapped on the membrane will bind the gold-antibody conjugate and unbound conjugate is removed from the membrane by the washing solution. The paper layer underneath the membrane absorbs excess liquid. Due to the bond gold particles, the membrane appears purple and the intensity of the colour is proportional to the albumin concentration present in the sample. All the urine samples were screened for urine protein by heat coagulation test [16]. The estimated Glomerular Filtration Rate (eGFR) was calculated by MDRD GFR equation based on creatinine and participant DM characteristics.

eGFR (mL/minute/1.73 m^2) = 186 x (Creatinine / 88.4)-1.154 x (Age)-0.203 x (0.742, if female) x (1.210, if black) [17].

STATISTICAL ANALYSIS

The results of the study were indicated as mean±SD. Data were analysed using SPSS version 18.0 and p-value of <0.05 was considered as the cut off level for significance. Independent t-test was used to compare between the groups. In addition, Chi square test was used to find out the relationship between serum hs-CRP and metabolic variables in the study subjects.

RESULTS

Demographic data, clinical characteristics and biochemical variables of the study subjects are shown in [Table/Fig-1]. The mean age of Type 2 diabetic subjects without any complications was 52.8±8.8 years (men: 51.5±9.1 years, women: 54.1±8.5 years). The mean age of the diabetic nephropathy subjects was 54.7±7.0 years (men: 54.8±7.2 years, women: 54.7±7.1 years). Among the cases, the gender representation varies widely in different age groups. For detailed analysis, age was grouped into different categories. The age and gender wise distribution of the study cases are shown in [Table/Fig-2].

Mean hs-CRP level of the diabetic and diabetic nephropathy cases were significantly high (p<0.05) as compared to the hs-CRP levels in controls. A significant difference was observed (p<0.05) in mean FBG level in diabetic nephropathy patients and Type 2 diabetic patients when compared with controls. A significant difference (p<0.05) in

Variables	Non-diabetic healthy controls (n=32)	Type 2 diabetic patients without complications (n=32)	Type 2 diabetic patients with nephropathy (n=32)
Age (years)	24.6 ± 2.0	52.8 ± 8.8	54.7 ± 7.0
BMI (kg/m^2)	22.2±3.7	24.6±5.7	23.3±4.7
SBP (mm Hg)	122.1±12.2	140.6 ± 18.4*	151.7 ± 7.92*
Waist-hip ratio	0.87 ± 0.05	0.92 ± 0.08*	0.93 ± 0.06*
hs-CRP (mg/L)	0.31 ± 0.47	2.53 ± 0.71*	5.15 ± 1.27*
Microalbumin (mg/L)	5.9 ± 1.17	14.8 ± 3.94*	158.9 ± 38.97*
FBG (mg/dL)	94.65 ± 5.87	169.96 ± 23.71*	228.18 ± 33.29*
PPBG (mg/dL)	127.84 ± 13.75	279.03 ± 45.35*	395.71 ± 46.99*
TC (mg/dL)	177.31 ± 12.10	205.71 ± 22.76*	234.68 ± 25.77*
Triglycerides (mg/dL)	122.78 ± 19.63	198.40 ± 22.31*	222.25 ± 32.31*
HDL-C (mg/dL)	54.90 ± 2.65	42.56 ± 3.30	34.56 ± 2.77
LDL-C (mg/dL)	97.85 ± 11.03	123.47 ± 20.22*	155.67 ± 24.52*
TC: HDL-C ratio	3.23 ± 0.23	4.86 ± 0.67*	6.84 ± 1.01*
Creatinine (mg/dL)	0.57 ± 0.11	1.36 ± 0.19	2.03 ± 0.30*
eGFR (mL/minute/1.73 m^2)	165.1 ± 42.7	52.5 ± 13.9*	32.4 ± 7.6*

[Table/Fig-1]: Demographic data, clinical characteristics and biochemical variables of the study subjects (Independent t-test).
* Significant (p <0.05)
hs-CRP: High Sensitivity C-Reactive Protein, FBG: Fasting Blood Glucose, PPBG: Post Prandial Blood Glucose, TC: Total Cholesterol, HDL-C: High Density Lipoprotein Cholesterol, LDL-C: Low Density Lipoprotein Cholesterol, eGFR: Estimated Glomerular Filtration Rate.

Age (years)	Type 2 diabetic patients without complications (n=32)						Type 2 diabetic patients with nephropathy (n=32)					
	Total		Male		Female		Total		Male		Female	
	n	%	n	%	n	%	n	%	n	%	n	%
31-40	4	12.5	3	75.0	1	25.0	1	3.1	1	100.0	0	0
41-50	8	25.0	3	37.5	5	62.5	8	25.0	3	37.5	5	62.5
51-60	13	40.6	6	46.2	7	53.8	13	40.6	7	53.8	6	46.2
61-70	7	21.9	4	57.1	3	42.9	10	31.3	5	50.0	5	50.0

[Table/Fig-2]: Age and gender wise distribution of the study cases.
* Expressed in number (n) and percentage (%)

mean PPBG level of diabetic nephropathy patients and diabetic patients as compared to controls were also observed. In this study, significant differences (p<0.05) were observed in mean TC, LDL-C and TG levels of diabetic patients and diabetic nephropathy patients as compared to non-diabetic subjects. A significant difference (p<0.05) in mean microalbumin level was observed in diabetic and diabetic nephropathy when compared to healthy controls. A significant difference (p<0.05) in mean creatinine level of diabetic nephropathy patients and no significant difference (p>0.05) in diabetic patients as compared to controls was also observed. The study also showed a significant decrease (p<0.05) in the mean level of eGFR in both diabetic and diabetic nephropathy subjects.

Association of hs-CRP with biochemical variables in Type 2 diabetic patients without any complications and with nephropathy are shown in [Table/Fig-3,4] respectively. The result shows a significant (p<0.05) relationship between hs-CRP and metabolic variables like Fasting Blood Glucose (FBG), Postprandial Blood Glucose (PPBG), Total Cholesterol (TC), Triglycerides (TG), Low Density Lipoprotein Cholesterol (LDL-C) and TC:HDL-C ratio in both diabetic and diabetic nephropathy subjects. Serum hs-CRP was also associated with serum creatinine concentration (p<0.05) in diabetic

Variables	Chi square test value	df	Sig. (2-tailed)
Microalbumin (mg/L)	14.891	3	0.002*
FBG (mg/dL)	8.631	2	0.013*
PPBG (mg/dL)	18.903	3	0.001*
TC (mg/dL)	7.158	1	0.007*
Triglycerides (mg/dL)	5.776	1	0.016*
HDL-C (mg/dL)	1.157	1	0.282**
LDL-C (mg/dL)	5.584	1	0.018*
TC: HDL-C ratio	7.997	3	0.046*
Creatinine (mg/dL)	0.922	1	0.337**
eGFR (mL/minute/1.73 m^2)	21.332	5	0.001*

[Table/Fig-3]: Association of hs-CRP with biochemical variables in Type 2 diabetic patients without any complications (Chi square test applied).
* Significant, ** Not significant
df: degree of freedom, FBG: Fasting Blood Glucose, PPBG: Post Prandial Blood Glucose, TC: Total Cholesterol, HDL-C: High Density Lipoprotein Cholesterol, LDL-C: Low Density Lipoprotein Cholesterol, eGFR: estimated Glomerular Filtration Rate.

Variables	Chi square test value	df	Sig. (2-tailed)
Microalbumin (mg/L)	10.633	4	0.031*
FBG (mg/dL)	13.431	4	0.009*
PPBG (mg/dL)	25.806	8	0.001*
Total Cholesterol (mg/dL)	15.458	4	0.004*
Triglycerides (mg/dL)	13.021	4	0.011*
HDL-C (mg/dL)	4.894	2	0.087**
LDL-C (mg/dL)	21.468	6	0.002*
TC: HDL-C ratio	29.321	4	0.001*
Creatinine (mg/dL)	10.014	4	0.04*
eGFR (mL/minute/1.73 m^2)	17.576	4	0.001*

[Table/Fig-4]: Association of hs-CRP with biochemical variables in Type 2 diabetic patients with nephropathy (Chi square test applied).
* Significant, ** Not significant
df: degree of freedom, FBG: Fasting Blood Glucose, PPBG: Post Prandial Blood Glucose, TC: Total Cholesterol, HDL-C: High Density Lipoprotein Cholesterol, LDL-C: Low Density Lipoprotein Cholesterol, eGFR: estimated Glomerular Filtration Rate.

nephropathy, but no association was observed in Type 2 diabetic patients without any complications. No significant relationship was observed between hs-CRP and HDL-C (p>0.05) in both diabetic and diabetic nephropathy subjects. A significant association was observed between hs-CRP and eGFR (p<0.05) in both diabetic and diabetic nephropathy subjects.

DISCUSSION

Our findings indicate that the concentration of serum hs-CRP was elevated in Type 2 diabetic and diabetic nephropathy patients than non-diabetic controls. There may be a significant relationship between hs-CRP and complications of Type 2 diabetes mellitus through the acute phase response. Stehouwer CD et al., Jager A et al., and Samia M et al., reported the relationship between inflammation, Type 2 diabetes mellitus and its complications [18-20]. A high level of inflammatory plasma proteins leads to the increased incidence of cardiovascular risk especially in Type 2 diabetic patients. An increased incidence of vascular complications of Type 2 diabetes mellitus has been observed among subjects with high levels of inflammatory markers [18,19]. Measurement of markers of inflammation might be helpful for the assessment of the vascular risk in Type 2 diabetic patients. Inflammation is linked to the pathogenesis of Type 2 diabetes mellitus. Insulin resistance and hyperglycemia are also promoting inflammation by increased oxidative stress and that may link diabetes mellitus to the development of atherosclerosis [20].

Serum hs-CRP is one of the factors as a biomarker for predicting cardiovascular events as serum hs-CRP is correlated with the cardiovascular risk and elevations of other biomarkers are found to be significantly associated with vascular events. It was found that study subjects with high CRP and low LDL-C were at higher risk than those in the low CRP and high LDL-C group. Even after adjustment for age, smoking, blood pressure, Type 2 diabetes mellitus, HDL-C and LDL-C levels, CRP remain as an independent prognostic risk factor. These observations indicate the need for physicians to consider CRP in one of the biomarkers [21]. The best characterized inflammatory biomarker is C-Reactive Protein (CRP). Sridevi D et al., found that the CRP levels are elevated in Type 2 diabetic patients with the metabolic syndrome and hence, hs-CRP is added as a diagnostic criterion for metabolic syndrome [22]. A study on hs-CRP levels and glycated haemoglobin by King DE et al., indicate the relationship between hs-CRP and glycaemic control [23].

Mahajan A et al. found a relationship between C-reactive protein and hyperglycemia in urban Northern Indian Type 2 diabetic patients which is an agreement with our study [24]. Lima LM et al., reported that hyperglycemia is an associated factor to the increased serum CRP levels in uncontrolled Type 2 diabetes subjects [11]. Study on inflammatory markers by Waheed P et al., showed the relationship between dyslipidemia, C-reactive protein and low-grade inflammation in diabetic subjects [25]. Mojahedi MJ et al., reported that microalbuminuria is accompanied by elevated hs-CRP, suggesting activation of inflammatory pathways in the progression of cardiovascular and renal disease in Type 2 diabetic patients which is an agreement with our findings [26]. The inflammatory response may be due to microvascular or macrovascular complications following Type 2 diabetes mellitus, although the exact mechanisms are still not well understood. Serum hs-CRP level is elevated in Type-2 diabetes and diabetic nephropathy in comparison with non-diabetic healthy controls. The results of the present study showed that the serum hs-CRP concentration is strongly related to Type 2 diabetes and diabetic nephropathy.

LIMITATION

A very small sample size is an evident limitation of this study which is due to the limited availability of data within the short duration of the study. In addition, the consent from the patients was not easy as many of them were unaware of the importance of the study and hence such cases were rejected to participate in the study. Randomized controlled trials in Type 2 diabetic patients with nephropathy and/or cardiovascular disease are recommended to generate more evidences to support the findings. Further research is required to observe whether there is a beneficial strategy to

reduce the risk of diabetic nephropathy in Type 2 diabetic patients with high levels of hs-CRP.

CONCLUSION

The main finding of this study is that high level of serum hs-CRP is strongly related to the metabolic variables and predictors of cardiovascular risk in Type 2 diabetic patients with and without nephropathy. The study strongly suggests the importance of measuring the level of serum acute-phase proteins in the diagnosis and early detection of complications of Type 2 diabetes mellitus. There may be an association between hs-CRP and complications through the acute phase response. The hs-CRP might be considered as a predictor or illness indicator for the development of diabetic nephropathy and cardiovascular disease in Type 2 diabetic patients.

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