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Study of Serum Highly Sensitive C-reactive Protein in Normotensive Diabetics and Hypertensive Diabetics

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Abstract

Diabetes mellitus and hypertension are known to increase markers of inflammation, ie, highly sensitive C-reactive protein [(hs)CRP], especially when they develop micro-albuminuria. A total of 70 patients (37 males and 33 females), all having diabetes mellitus, according to ADA criteria and micro-albuminuria in morning spot urine sample were recruited in the study. They were randomised into 2 groups. Group A comprised hypertensive (34 patients) and Group B (36 patients) normotensive individuals. (hs)CRP level was assessed in all patients by routine assay. (hs)CRP value of >3 mg/L was observed in 28.57% patients of group A and 12.86% patients of group B (p=0.004). So diabetic patients with micro-albuminuria had more frequent association with increased marker of inflammation in the hypertensive group compared to those without hypertension.

Keywords: Micro-albuminuria; C-reactive protein; Diabetes; Hypertension

Introduction

Diabetes mellitus and hypertension are two of the most common diseases in Westernized, industrialized civilizations, and the frequency of both diseases increases with increasing age [1]. Diabetes is fast gaining the status of a potential epidemic in India with more than 62 million diabetic individuals currently diagnosed with the disease [2]. Although diabetes mellitus is associated with the considerably increased cardiovascular risk, the presence of hypertension in the diabetic individual markedly increases morbidity and mortality. As per the data drawn from death certificates, hypertension has been implicated in 44% of deaths related to diabetes, and diabetes is involved in 10% of deaths related to hypertension [3]. It has been estimated that 35-75% of diabetic complications can be attributed to hypertension. In contrast, the absence of hypertension is the usual finding in long-term survivors of diabetes [4]. Thus, the coexistence of these two diseases likely contributes substantially to overall mortality in industrialized societies. Despite the critical importance of the coexistence of these two diseases, much information regarding their interaction remains unclear and controversial. Nevertheless, much information of theoretical and practical relevance is available, and there is considerable on-going research exploring the relation between carbohydrate intolerance and hypertension.

It is not our intent to compile an exhaustive survey of the interrelation of hypertension and diabetes mellitus. Rather, we will emphasize selective issues that we believe are timely and have recently attracted increased attention and investigative interest. First, we will examine and highlight newer avenues of investigation, focusing on the role of abnormalities in vascular smooth muscle and endothelial damage as a part of inflammatory process, such as acute phase reactant like C Reactive Protein. It is well appreciated both that coexisting hypertension exacerbates diabetic nephropathy.

C-reactive protein ((hs)CRP) is an acute phase reactant. Its normal level is 0.08-3.1 mg/L by high sensitive assay. High (hs)CRP level(>3 mg/L) is associated with potentiating of atherosclerosis and its complications. The American Heart Association and U.S. Centres for Disease Control and Prevention have defined risk groups as follows:

- Low risk: less than 1.0 mg/L
- Average risk: 1.0 to 3.0 mg/L
- High risk: above 3.0 mg/L

In the present study we measured (hs)CRP level was measured in 70 patients, all having diabetes mellitus and micro-albuminuria. They were randomised into 2 groups. Group A comprised hypertensive (34 patients) and group B normotensive (36 patients) individuals. The aim of the study was to compare (hs)C-reactive protein in Normotensive Diabetics and Hypertensive Diabetics with microalbuminuria and to study correlation of microalbuminuria and (hs)C-reactive protein levels in Normotensive Diabetics and hypertensive diabetics.

Materials and Methods

This Cross-sectional study, 'Highly sensitive C-Reactive Protein in normotensive diabetics and hypertensive diabetics with microalbuminuria' was carried out in tertiary care centre over a

period of two years from November 2011 to November 2013 after prior approval from the institutional ethics committee.

Sample size

A total of 70 subjects were required for completion of study.

Cases

All patients attending the medicine OPD or admitted in wards were screened for inclusion and exclusion criteria.

Inclusion criteria

All patients of type 2 diabetes mellitus of either sex above 30 years of age group were included.

Both diagnosed cases of diabetes mellitus on treatment and newly diagnosed cases were included. Criteria for diagnosis of diabetes mellitus (WHO)

- Symptoms of diabetes plus random plasma glucose concentration>200 mg/dl (11.1 mmol/L). Random was defined as anytime of a day without regards to time since last meal. The classic symptoms of diabetes used were polyuria, polydipsia and unexplained weight loss.
- Fasting plasma glucose>126 mg/dl (7.0 mmol/L). fasting was defined as no caloric intake for last 8 hours or
- 2 hour Post glucose>200 mg/dl during Oral Glucose Tolerance Test. The test was performed as described by WHO, using glucose containing the equivalent of 75 grams of anhydrous glucose dissolved in water.

Type 2 diabetes mellitus was diagnosed by

- Age of onset (>30 years).
- History of taking oral hypoglycemic drugs.
- No history of ketoacidosis.
- Patients giving history of type 2 diabetes at time of interview.

Exclusion criteria

- Patients with overt albuminuria (>300 mg), congestive cardiac failure, urinary tract infection, pregnant patients, patients confined to bed for more than 2 weeks.
- Patients with other causes of microalbuminuria like heavy metal poisoning, connective tissue disorder and chronic use of NSAIDs.
- History of liver, kidney, acute illness and thyroid diseases, anemia, haemochromatosis which are known to influence hsCRP levels.
- Gestational diabetes.
- Women on hormone replacement therapy which are known to influence serum hsCRP levels.
- Patients with infection, overt nephropathy, pre-existing kidney/prostatic disease, congestive heart failure, pregnancy, oestrogen, etc., were excluded from the study.
- Patients not willing to participate in study.

Method of urine examination

First morning urine specimen was collected in a clean container free from detergent. Specimen was tested within 12 hours of collection. Turbidimetric immunoassay method for determination of microalbuminuria was used.

A diagnosis of microalbuminuria was made when the ratio of urinary albumin to creatinine was 30-300 mcg/mg and macroalbuminuria was diagnosed if the ratio was >300 mcg/mg. Normoalbuminuria was said to exist if the ratio was <30 mcg/mg. All patients with normoalbuminuria and macroalbuminuria were excluded from the study.

Patients with microalbuminuria were further divided into two groups, hypertensives (Group A) and normotensives (Group B).

Hypertension was defined according to Joint National Committee (7^{th} JNC) criteria. Patients were considered as hypertensive if either systolic blood pressure was >140 mmHg or diastolic blood pressure was >90 mmHg or if the patient was a diagnosed case of hypertension on antihypertensive medications.

All patients were subjected to detailed history, clinical examination and laboratory investigations which includes

- Glycosylated haemoglobin (Hba1c)
- Total cholesterol
- Serum triglycerides
- LDL
- HDL
- VLDL
- BMI
- hsCRP

Measurement of (hs)C-reactive Protein

The 3 ml blood sample for (hs) CRP was taken from cubital vein and was tested immediately or was stored at 2-8˚C and tested within 8 hours, for (hs)CRP measurement. The hsCRP was measured quantitatively by turbid metric test using kits from SPINREACT, Spain.

A value of >3 mg/l was considered as high. (hs)CRP level was measured in all patients and prevalence of (hs)CRP positivity was assessed in both the groups.

Statistical Analysis

The data was collected and analyzed at the end of the study. Continuous variables (age, anthropometry, biochemical and clinical) were presented as Mean ± SD. Categorical variables were expressed in percentage. Anthropometry, biochemical and clinical parameters were compared by un-paired t-test. Pearsons Chi square statistics was used for comparison of categorical variables. Pearsons correlation coefficient was used to assess the correlation between various parameter. Chi square test for linear trend was applied whenever necessary. Odds ratio (OR), 95% confidence interval were estimated for risk factors multiple logistic regression analysis was performed to identify

independent predictors of (hs)CRP. p<0.05 was considered statistically significant. Statistical software SPSS version 17.0 was used for data analysis.

Observations

Comparison of the two groups with respect to baseline data in Group A and Group B are depicted in Tables 1 and 2.

Table 1: Baseline characteristics of patients in group A.

Variables	No. of patients	Maximum	Minimum	Mean ± SD
Age (years)	34	78	46	56.91 ± 7.89
Duration of DM (years)	34	9	3	7.53 ± 1.93
BMI (kg/m^2)	34	36.2	19.7	28.16 ± 4.99
Systolic BP (mmHg)	34	178	156	165.29 ± 6.02
Diastolic BP (mmHg)	34	100	88	95.23 ± 3.22
FBS (mg/dl)	34	289	134	188.88 ± 48.47
Hba1c (%)	34	10.8	8	8.93 ± 0.79
Total Cholesterol (mg/dl)	34	253	111	179.67 ± 36.57
Sr. Triglycerides (mg/dl)	34	531	100	237.82 ± 119.58
HDL (mg/dl)	34	45	25	35.99 ± 6.30

Table 2: Baseline characteristics of patients in group B.

Variables	No. of patients	Maximum	Minimum	Mean ± SD
Age (years)	36	68	45	52.5 ± 6.54
Duration of DM (years)	36	10	5	7.22 ± 1.68
BMI(kg/m^2)	36	34.8	18.7	24.46 ± 3.82
Systolic BP(mmHg)	36	140	110	129.05 ± 6.98
Diastolic BP(mmHg)	36	88	68	74.94 ± 5.33
FBS (mg/dl)	36	194	89	151.55 ± 28.77
Hba1c (%)	36	8.5	7	7.91 ± 0.39
Total Cholesterol (mg/dl)	36	238	110	158.38 ± 28.95
Sr. Triglycerides (mg/dl)	36	397	80	165.77 ± 67.97
HDL (mg/dl)	36	45	30	39.61 ± 3.96

Gender wise distribution of patients is depicted in Table 3.

Table 3: Gender wise distribution of patients.

Gender	Group A (n=34)	Group B (n=36)	Total	Chi-square p-value
Male	16 (22.86%)	21 (30%)	37	0.89
Female	18 (25.71%)	15 (21.43%)	33	p-value=0.34
Total	34 (48.57%)	36 (51.43%)	70	NS, p>0.05

Out of total 34 patients of group A, 20(28.57%) showed (hs)CRP-positivity and among total 36 patients of group B, 9(12.86%) showed (hs)CRP-positivity. The rates-corrected chi-square values was 8.24 and p-value was 0.004 (Table 4).

Table 4: Distribution of patients according to (hs) CRP Positivity.

CRP	Group A n=34	Group B n=36	Chi-square p-value
Positive (≥ 3 mg/l)	20 (28.57%)	9 (12.86%)	8.24
Negative (<3 mg/l)	14 (20%)	27 (38.57%)	p=0.004
			S, p<0.05
Total	34 (48.57%)	36 (51.43%)	

Thus group A patients had more significant (hs)CRP positivity than group-B. So, diabetic patients with micro-albuminuria have a more significant association with (hs)CRP in hypertensive patients than in diabetic patients with normal blood pressure.

We also looked into the association of micro-albuminuria with (hs)CRP in hypertensive diabetic and normotensive diabetic patients (Table 5). It showed that patients with hsCRP positivity in hypertensive (Group A) had higher mean microalbuminuria as compared to normotensive (Group B).

Table 5: Association of (hs) C-RP positivity with microalbuminuria.

Microalbuminuria (mcg/mg)	Group A (n=20)	Group B (n=9)	Value-t	p-value
Mean+SD	257.50 ± 9.32	238.88 ± 39.10	2.04	0.041 S, p<0.05

- Table 6 shows the distribution of patients according to BMI in group A and B. There was no significant difference in BMI in both the groups (p-value=0.74). But when compared in hsCRP positive groups, BMI was significantly higher in hypertensive group compared to normotensive group (Table 7). Thus showing the positive association between BMI and hsCRP.
- Table 8 has shown the association of hsCRP and dyslipidemia. It was observed that patients with hsCRP positivity in hypertensive group have significant dyslipidemia compared to normotensive groups.
- Poor glycemic control, as indicated by higher Hb1ac values has positive association with hsCRP as shown in Table 9.
- Multiple logistic regressions (Table 10) have shown Age, Hypertension, poor glycemic control and BMI as independent predictors of hsCRP positivity.

Discussion

Diabetes mellitus and hypertension are two of the most common diseases in Westernized, industrialized civilizations, and the frequency of both diseases increases with increasing age. Although diabetes mellitus is associated with the considerably increased cardiovascular risk, the presence of hypertension in the diabetic individual markedly increases morbidity and mortality.

Several epidemiological and clinical studies have demonstrated that the presence of microalbuminuria is an independent and strong predictor of cardiovascular mortality and morbidity in patients with diabetes mellitus (DM) [5-8]. It has been shown that microalbuminuria is a marker of vascular damage and atherosclerosis [6,9]. On the other hand, high serum levels of C-reactive protein (CRP) is associated with complications of atherosclerosis such as myocardial infarction and stroke [9-11]. Hence, serum CRP might be potentially a marker that C-Reactive Protein and Albuminuria in Diabetic Patients is associated with a higher risk of mortality in atherosclerotic patients [12]. In one study, it was reported that elevated serum CRP level increased diabetic risk up to 2.7 times [13]. Microalbuminuria is also associated with endothelial damage [14,15]. Therefore, it can be anticipated that albuminuria level can be associated with higher levels of serum CRP and activation of inflammatory pathways in progression of renal and cardiovascular atherosclerotic diseases can reflect in the CRP level [16]. Accordingly, we carried out a study on patients with type 2 DM to investigate the relationship of serum CRP level and microalbuminuria. We also aimed to study the association of hsCRP in diabetic patients with hypertension and normotension along with various risk factors.

This study showed that the hypertensive group (group A) had a significant association with (hs)CRP-positivity (Chi-square=8.24, p=0.004). Stuveling et al. [17] had also shown that the association between micro-albumainuria and (hs)CRP was more significant (p<0.0001) in subjects with high mean arterial pressure. They concluded that BP positively modified the relationship between micro-albumainuria and (hs)CRP.

Tsioufis et al. [18] had shown that micro-albuminuria is accompanied by increase in (hs)CRP level in the setting of hypertension, reflecting a diffuse atherosclerotic process. So, the findings in the present study are consistent with the above-mentioned study results. Older age was significantly associated with (hs)CRP-positivity in both the groups. This is also supported

by studies, done by Stuveling et al. [17], Tsioufis et al. [18] and Nakamura et al. [19]. BMI was also higher in (hs)CRP (+)ve patients in both the groups in the present study (Table 6).

Stuveling et al. [17], Tsioufis et al. [18] and Laaksonen et al. [20] have also shown similar results in their studies.

Table 6: Distribution of patients according to BMI (kg/m^2).

BMI	Group A (n=34)	Group B (n=36)	Chi-square p-value
<25(kg/m^2)	15 (21.43%)	17 (24.29%)	
≥ 25(kg/m^2)	19 (27.14%)	19 (27.14%)	0.1 p-value=0.74 NS, p>0.05
Total	34 (48.57%)	36 (51.43%)	
Mean	28.16	24.46	
SD	4.99	3.82	

In this study, both the groups showed significant association of dyslipidaemia (Table no.7) with (hs)CRP-positivity (Table 8). Stuveling et al. [17] have shown that high (hs)CRP-level is associated with high total cholesterol level and high incidence of CAD (Table 8).

Table 7: Association of (hs) C-RP positivity with BMI(kg/m^2).

BMI (kg/m^2)	Group A (n=20)	Group B (n=9)	OR	95% CI	2κ-value	p-value
≥ 25	15(75%)	5(55.56%)	28	1.04-7.95	4.33	0.034
<25	5(25%)	4(44.44%)				S, p<0.05

Tsioufis et al. [18] have also shown that (hs)CRP-positivity was associated with high total cholesterol and LDL-cholesterol level. In a study done by Laaksonen et al. [20], (hs)CRP +ve individuals had high incidence of dyslipidaemia and cardiovascular disease.

Table 8: Association (hs) C-RP positivity with TC, Sr. TG, Sr. LDL and Sr. HDL.

Lipid Profile	(mg/dl)	Group A (n=20)	Group B (n=9)	OR	95% CI	2κ-value	p-value
TC	>200	14(70%)	5(55.56%)	1.83	1.02-3.28	4.2	0.04 S, p<0.05
	≤ 200	6(30%)	4(44.45%)				
TG	>150	13(65%)	5(55.56%)	0.44	0.24-0.77	8.08	0.04 S, p<0.05
	≤ 150	7(35%)	4(44.45%)				
LDL	>100	11(55%)	3(33.33%)	2.48	1.39-4.40	9.82	0.001 S, p<0.05
	≤ 100	9(45%)	6(66.67%)				
HDL	>40	6(30%)	4(44.45%)	2.97	1.65-5.31	13.79	<0.001 S, p<0.05
	≤ 40	14(70%)	5(55.56%)				

However, certain studies have not found any significant association of hsCPR with hypertension, body mass index and dyslipedemia [21].

On multiple logistic regression (Table 9), we found age, hypertension, higher BMI and poor glycemic control as independent predictors for hsCRP.

We did not found duration of diabetics as independent risk factor for hsCRP. This finding may be attributed to wrong information and or uncertainty about duration of diabetics to subjects and needs further studies.

Table 9: Association of (hs) C-RP positivity with Glycosylated Hemoglobin (Hba1c).

Hba_1c (%)	Group A (n=20)	Group B (n=9)	OR	95% CI	2x-value	p-value
>7	14(70%)	5(55.56%)	1.83	1.02-3.28	4.2	0.04 S, p<0.05
≤ 7	6(30%)	4(44.45%)				

So, to conclude, the present study revealed that in diabetic patients, micro- albuminuria has a significant association with (hs)CRP and the association is stronger in hypertensive patients than in normotensive patients.

Table 10: Multiple Logistic Regression Analysis for prediction (hs) C-RP.

RISK FACTORS	t-value	Adjusted Odd's Ratio	p-value	95% Confidence Interval for B	
				Lower Limit	Upper Limit
Age	0.241	2.709	0.009,S, p<0.05	0.028	0.004
Duration of DM	0.081	0.823	0.413,NS, p>0.05	0.075	0.031
Hypertension	0.072	3.549	0.006, S, p<0.05	0.329	0.187
BMI	0.029	2.303	0.004, S, p<0.05	0.017	0.023
Hba1c	0.347	2.875	0.003, S, p<0.05	0.155	0.028

This study supports the notion that raised (hs)CRP in association with micro-albuminuria may be a risk factor for cardiovascular disease in addition to the conventional cardiovascular risk factors.

It also supports the notion that presence of hypertension with diabetics potentiates inflammatory process more compared to those having normotension with diabetes, as indicated by hsCRP and hsCRP should be considered as routine tool in evaluating and predicting cardiovascular risk in selected population groups. Thus, there is need to explore newer avenues in this respect and emphasizes the need for higher studies.

Limitations of the Study

There were few limitations to the study results

In the present study, microalbuminuria was based on a single urinary spot collection; the ADA recommends that positive result be reconfirmed with repeat tests over a period of 3-6 months. However, this may not change the inferences drawn as most epidemiological studies have followed the methodology of single urine sample measurements.

Besides, we did not confirmed the presence of type 2 diabetes mellitus by studying auto-antibodies; hence it is difficult to say that the study group consisted of only type 2 diabetic patients only.

The (hs) C-Reactive Protein affecting/lowering effect of anti-diabetic drugs, anti-hypertensive drugs and statins were not considered in the present study.

As it was a hospital based study, the true prevalence in the population can neither be assessed nor extrapolated.

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