Association of Serum hsCRP with Lipid Profile in Patients with T2DM attending a Tertiary Care Hospital in Bangladesh

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Abstract

Background: Diabetes is a major healthcare problem due to its prolonged effect of complications deteriorating our quality of life. Chronic inflammation has now become the major concern in developing atherosclerotic changes leading to cardiovascular complications in diabetes. C-reactive protein, a sensitive marker of low grade systemic inflammation may determine oxidative stress on endothelium in diabetic patients. As serum hsCRP and lipid profile show related process for the same disease, these variables are expected to have strong relations in type 2 diabetes mellitus.

Methods: A cross sectional analytical study was conducted on 119 subjects, who were selected according to the criteria from the Outpatient department of BIRDEM general hospital. Relevant biochemical parameters such as fasting blood glucose, blood glucose 2 hours after breakfast, HbA_1c, lipid profile and hsCRP were measured and included in the questionnaire along with anthropometric measurements.

Result: Participants with higher hsCRP had increased levels of FBG, blood glucose 2 hours after breakfast and HbA_1c. Hypertriglyceridemia (32.1% vs. 73%, $p<0.001$), increased LDL-C (47.5% vs. 85%, $p<0.05$) and lower HDL-C were found in higher hsCRP. hsCRP had positive association with total cholesterol ($r=0.306$, $p<0.01$), triglyceride ($r=0.428$, $p<0.05$) and LDL-C ($r=0.22$, $p<0.05$). hsCRP showed linear association with lipid parameters individually after adjusting confounding variables like age, gender, BMI and parameter for glycemic control, HbA_1c.

Conclusion: The study showed significant association of hsCRP with glycemic index and lipid profile in diabetic subjects. Higher hsCRP indicating subclinical inflammation was found significantly associated with poor glycemic control and dyslipidemia mainly hypertriglyceridemia. It was well understood that hsCRP could be used as an additional marker along with lipid profile to see prognosis of T2DM.

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Introduction

T2DM is primarily defined by hyperglycaemia giving rise to risk of micro and macrovascular damage.[1] In country profile of Bangladesh World Health Organization found 8% prevalence of diabetes.[2] This decreased insulin secreting disease has an asymptomatic phase of minimum 4-7 years between the actual onset of persistent hyperglycemia and clinical diagnosis. This latent period aggravates low grade systemic inflammation in T2DM due to insulin resistance.[3,4,5] As chronic inflammation enhances oxidative stress on the endothelium, easily available inflammatory marker like hsCRP may be associated with poor glycemic control.[6,7,8] C-reactive protein (CRP), the first acute phase reactant is a sensitive marker of inflammation, produced and released by liver under the stimulation of cytokines like tumor necrosis factor-α and inter-leukins1 and 6.[9, 10]

High CRP also releases tissue factor from macrophages, activates the complement system and causes the aggregation of LDL-cholesterol and VLDL-cholesterol by binding with them.[11] In separate studies association of CRP with glycemic control and lipidemic status have been found among diabetic Bangladeshis.[12, 13] But the combined effects of dyslipidemia and hsCRP in T2DM is still required for further observation.[12]

We therefore undertook this study on hsCRP levels in T2DM patients to observe its association with the lipidemic status and to find out whether hsCRP could be an effective marker for early detection of cardiovascular complications in T2DM.

Methods

Study place and population

In this cross sectional study patients with previously diagnosed T2DM according to the WHO diagnostic criteria[2] participated from the outpatient department of BIRDEM general hospital over a period of one year. After explanation of total study informed written consent was taken from each participant. T2DM with chronic disease (liver and kidney diseases, arthritis), acute infection (urinary and respiratory tract infection) and pregnancy and with complications were excluded. The study was approved by the ethical committee of BADAS.

Study procedure

Anthropometric measurement was done including height, weight, waist-hip ratio and body mass index was calculated. 8 ml of venous blood was collected from study subjects after an overnight fasting of 10-12 hours. From this sample, 3ml was delivered in a plain test tube for estimation of fasting blood glucose, lipid profile and 3 ml into EDTA tube for estimation of HbA_1c. Rest 2ml blood was collected into another plain vaccutainer for measuring hsCRP. 2 hours after breakfast 3 ml blood was collected for blood glucose 2 hours after breakfast.

Separated serum was stored in refrigerator at -20° c. All biochemical measurements were carried out on the different days at scheduled time at the clinical biochemistry section and immunology section of laboratory department, BIRDEM General Hospital. Blood glucose level was measured using the glucose hexokinase method. HbA_1C was measured by high performance liquid chromatography (HPLC) method by Variant™ II Turbo Kit-2.0. Serum total cholesterol, triglyceride concentrations were measured by enzymatic end point technique using Dimension® clinical chemistry system (Siemens Healthcare Diagnostics Inc. USA) using Flex reagent cartridge. HDL cholesterol levels were measured by the fully automated reagent format of Dimension® clinical chemistry system (Siemens Healthcare Diagnostics GmbH, Germany) and LDL

cholesterol concentrations were calculated by Friedewald's formula.[14] High sensitivity CRP was detected by immunonephelometric method using BNProspec® system (Siemens Healthcare Diagnostics Inc. USA).

Operational definition

The Centers for Disease Control and Prevention and the American Heart Association (CDC/AHA) in January of 2003 issued the clinical guidelines for hs-CRP for global risk prediction and suggested that levels of hs-CRP at <1, 1 to 3, and >3 mg/L to represent low, moderate and high vascular risk.[15]

Statistical analysis was performed with the help of SPSS (20) version. Descriptive statistics were presented as mean±SD for normally distributed data. Statistical significance of differences between the values was assessed using t-test and chi square test. Correlation analysis was done by using spearman's correlation. Regression analysis was done.

Results

Demographic characteristics of study population were shown in table 1. 53.8% had family history of DM and 46.2% of the participants could not provide proper family history.

Table I: Demographic characteristics of the study participants (n=119)

Variables	Frequency	Percentage (%)
Gender		
Male	43	36.1
Female	76	63.9
Age		
≤50 years	57	47.9
>50 years	62	52.1
Smoking history		
Smoker	4	3.4
Nonsmoker	100	84.0
Recently past smoker	4	3.4
Past	11	9.2
Family history of DM		
Yes	64	53.8
No	55	46.2

Total study population was divided into two groups using hsCRP cut-off at > 3mg/L. 53.8% T2DM participants had high hsCRP. BMI (25.19±2.64 vs. 26.89±3.76, p<0.05), FBG (6.94±1.18 vs. 8.85±2.44, P< 0.001), blood glucose 2 hours after breakfast (9.88±2.2 vs. 12.81±4.02, P< 0.001) and HbA_1c (5.72±1.21 vs. 7.47±1.51, P< 0.001) were significantly higher in subjects with higher hsCRP (Table II). Aberrations in lipid profile were more in diabetic subjects with higher hsCRP (Table III). hsCRP had

significant association with total cholesterol (164.35±39.88 vs. 188.88±36.60, p< 0.05), LDL-Choesterol (98.78±24.1 vs. 114±33.42, p< 0.05) and triglyceride (154.84±61.13 vs. 208±74.7, p< 0.001). HDL-cholesterol was significantly less in high risk group of hsCRP than that of normal group of hsCRP (44.73±7.88 vs. 39.49±10.02, p< 0.05).

Table II: Clinical characteristics between normal and raised hsCRP of study population (n=119)

Variables	Normal hsCRP (n=55) Mean±SD	High hsCRP (n=64) Mean±SD	p- value
Age (years)	52.13±9.26	51.28±8.7	0.609
Duration of DM (Years)	8.35±4.9	7.93±5.49	>0.05
BMI (kg/m^2)	25.19±2.64	26.89±3.76	0.006
Waist-hip ratio	0.94±0.08	0.93±0.07	0.403
SBP (mm of Hg)	124.33±17.9	122.17±12.81	0.447
DBP (mm of Hg)	79.91±9.15	80.77±9.07	0.610
FBS (mmo/L)	6.94±1.18	8.85±2.44	<0.001
2h-ABF (mmol/L)	9.88±2.2	12.81±4.02	<0.001
HbA_1c (%)	5.72±1.21	7.47±1.51	<0.001

Independent t-test was done

Table III: Variations of lipid profile between two groups of hsCRP (n=119)

Variables	Normal hsCRP (n=55) Mean±SD	High hsCRP (n=64) Mean±SD	p- value
TC (mg/dl)	164.35±39.88	188.88±36.60	.001
LDL-C (mg/dl)	98.78±24.1	114±33.42	.007
HDL-C (mg/dl)	44.73±7.88	39.49±10.02	.002
TG (mg/dl)	154.84±61.13	208±74.7	.0001

Independent t-test was done

hsCRP was found significantly correlated with total cholesterol (r=0.306, p<0.01), triglyceride level (r=0.428, p<0.05) and LDL-cholesterol (r=0.22, p<0.05) in diabetics (table 4). Significant negative correlation was found between hsCRP and HDL-cholesterol (r=-0.245, p<0.01) in diabetics. It was also found significant positive association of hsCRP with FBG (r=0.447, p<0.001), blood glucose 2hours after breakfast (r=0.413, p<0.01) and HbA_1c (r=0.638, p<0.01).

Table IV: Correlation between hsCRP and clinical and biochemical variables of participants

Variables	r	p-value
Age (years)	0.095	>0.05
BMI (kg/m2)	0.280	<0.01
FBG (mmol/L)	0.447	<0.001
ABF (mmol/L)	0.413	<0.01
HbA1c (%)	0.638	<0.01
TC (mg/dl)	0.306	<0.01
TG (mg/dl)	0.428	<0.05
LDL-C (mg/dl)	0.220	<0.05
HDL-C (mg/dl)	-0.245	<0.01

Spearman's correlation; r=Correlation Coefficient.

hsCRP showed linear association with lipid parameters individually after adjusting confounding variables like age, gender, BMI and parameter for glycemic control, HbA_1c (table 5). 1 unit increase of hsCRP showed 0.437, 0.358 and 0.325 unit increase in triglyceride (β=0.437, p=0.001), total cholesterol (β=0.358, p<0.01) and LDL-C (β=0.325, p<0.01) respectively. But when hsCRP increased 1 unit, HDL cholesterol decreased by 0.358 unit (β=-0.358, p<0.01).

Table V: Regression analysis of hsCRP with lipid profile

Factors	Triglyceride		Total Cholesterol		LDL-C		HDL-C	
	β	p-value	β	p-value	β	p-value	β	p-value
HbA_1c	0.109	p>0.05	.047	p>0.05	-0.034	p>0.05	-0.038	p>0.05
hsCRP	0.437	p<0.001	.358	p<0.01	0.325	p<0.01	-0.358	p<0.01
Gender	-0.220	p<0.01	-.201	p<0.01	-0.031	p>0.05	0.345	p<0.001
Age	0.056	p>0.05	-.058	p>0.05	0.128	p>0.05	0.031	p>0.05
BMI	0.014	p>0.05	-.018	p>0.05	-0.057	p>0.05	-0.007	p>0.05

Discussion

This study showed association of hsCRP with glycemic status and lipid profile. Earlier it was reported that low-grade systemic inflammation exists in type 2 diabetes mellitus and is positively related with dyslipidemia.[16]

In our observational study more than half (53.8%) participants had high hsCRP in this population. Our obtained result is comparatively similar to the earlier work that emphasizes the association of hsCRP level with anthropometric variables and glycemic status of type 2 diabetes mellitus in Jordan.[17] Body mass index and hsCRP were significantly associated; though this association is independent of other parameters strongly associated to BMI such as age, gender, education and glycemic status. Elevated hsCRP had significantly positive association with BMI (p=0.006) which is also confirmed by Han et al 2002[18] Ramtanu et al 2013[8] and Anju et al 2015.[19]

A recent study estimated that one-third of type 2 DM cases can be associated with elevated serum CRP.[20] Elevated hsCRP levels were significantly associated with poor glycemic control which was shown in terms of HbA_1c (p<0.001), fasting blood glucose (p<0.001) and blood glucose 2 hours after breakfast (p<0.001). Previous study on sub continental Asians supports a strong association between hsCRP and HbA_1c and indicates hsCRP as an additional marker for better glycemic control.[16] King et al (2003) also confirmed linear association of hsCRP with HbA1c (p<0.05).[21] In Bedford survey positive association of hsCRP with FBG and HbA_1c is explained by the fact that inflammation is the principal state of insulin resistance observed in obesity and diabetes mellitus.[7]On the contrary Anju V et al have not found any significant association between CRP (and other inflammatory markers) and glycemic profile.[19]

A study on same population was done where elevated serum CRP was expressed >6 mg/L.[13] But we measured high sensitivity CRP (high CRP >3 mg/L) following the international guideline.[14,15] The relation of hsCRP with fasting plasma glucose and HbA_1c observed is similar to the previous studies of Pradhan and Ali.[8, 22, 23]

As the inflammatory mechanisms are involved both in diabetes and atherosclerosis, CRP levels tend to be increased in type 2 diabetes mellitus.[24] Literature on individual effects of dyslipidemia and inflammation on diabetes is available but more data is required on the combined effects of dyslipidemia and inflammation in diabetics.[12, 25]

In the present study we found positive association between hsCRP and triglycerides ((p<0.001) which was in tune with other studies presented by Ramtanu et al (2013) and Löwbeer et al (2015).[8, 26] On the contrary nonsignificant association has been shown between serum TG and hsCRP (p>0.05) by

Ali et al in 2015.[23] In this study significantly higher LDL cholesterol ($p<0.05$) was observed in individuals with higher hsCRP. In the present study, subjects with abnormal hsCRP had higher proportion of hypercholesterolemia ($p=0.001$). Similar result has been shown in the study of Amanullah S et al in 2010.[17] A negative association between hsCRP and HDL cholesterol ($p<0.05$) was observed in female participants of current study. In this study levels of total cholesterol, triglyceride and LDL cholesterol were significantly higher in high risk group of hsCRP and HDL cholesterol was significantly lower in females with high hsCRP which is consistent with previous study of Waheed et al in 2009.[16] This association may be explained by the fact that insulin resistance is invariably associated with release of excessive free fatty acids due to inhibition of lipoprotein lipase and stimulation of hormone-sensitive lipase.[27]

A good number of studies have been done on western populations showing the association of low grade systemic inflammation with diabetes mellitus.[28] There are few studies of hsCRP in subcontinent, despite being a very high risk group of diabetes.[22] Prospective studies should be conducted to determine the direction of this association.

Conclusion

In conclusion, the present study showed a strong association of hsCRP with glycemic profile and lipid profile in diabetic subjects. Higher hsCRP indicating subclinical inflammation was found significantly associated with hyperglycemia and dyslipidemia. It was also observed that higher BMI was associated with high risk group of hsCRP which explains the association of obesity with poor glycemic control and abnormal lipid profile. It was well understood that high risk group of hsCRP were more prone to develop diabetic complications.The current study demonstrated that higher HbA_1c and dyslipidemia mainly triglyceridemia were significantly associated with elevation of CRP. These results implied a significant relation between inflammation and glycemic control in people with established diabetes. Prospective studies should be conducted on this association that might help in assessing atherosclerotic complications and ease the lifestyle of diabetic adults. This study supported the use of hsCRP as an additional marker along with glycemic and lipidemic index in T2DM individuals for their betterment.

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