Utility of hsCRP as an add on to Lipid Profile for Cardiovascular Risk Stratification in Adults with Type 2 Diabetes Mellitus

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Abstract
Introduction: Dyslipidemia is common in Diabetes and is predictive of cardiovascular events. But, myocardial infarction in the setting of normal lipids levels is not uncommon. hsCRP has been studied elaborately and is found to be a stronger predictor of heart attack and stroke than LDL cholesterol. We conducted this study to observe the level of hsCRP in adults with Type 2 Diabetes and its association with lipid parameters. **Methods:** It is a cross sectional study including 168 Type 2 Diabetes patients conducted in department of biochemistry and internal medicine at B.P. Koirala Institute of Health Sciences, Dharan, Nepal for duration of one year. The ethical clearance was taken from the institutional ethical review board and patients were enrolled after taking informed consent. Venous blood was collected and serum lipid profile and hsCRP were measured. **Results:** The means±SD for age, TC, HDL-C, LDL-C and HDL/LDL ratio of patients were 52.2±11.9 years, 182.9±41.9 mg/dl, 41.6±8 mg/dl, 94.9±20 mg/dl, and 0.47±0.18 respectively. The medians of TG and hsCRP were 152.5 (109, 195) mg/dl and 1.9 (0.9, 2.8) mg/dl respectively. hs-CRP was found to have significant positive correlation with TC (r=0.286), LDL (r=0.652) and TG (r=0.299) and significant negative correlation with HDL (r= -0.614) and HDL/LDL ratio (r= -0.646). Only 33% of patients were categorised as having increased CVS risk according to high LDL levels but altogether 75% of patients had increased CVS risk according to hsCRP levels. **Conclusion:** hs-CRP can be considered as an add on to lipid profile while predicting CVS complications in patients with Type 2 Diabetes Mellitus in our population.

Keywords: Dslipidemia, hsCRP, Lipid Profile

Introduction
Pathogenesis of type 2 diabetes mellitus is much more complicated and involves many pathways leading to insulin resistance. A large body of data has recognized potential role of long standing inflammation in the causation Type 2 Diabetes[1,2]

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as well as in the progression of atherosclerosis and atherothrombosis.[3,4] High-sensitivity C-reactive protein (hsCRP) is an acute-phase response protein that is considered both a marker of inflammation and a predictor of cardiovascular events, including myocardial infarction, stroke, peripheral arterial disease, and sudden cardiac death.[5,6,7] Prospective studies have found high CRP levels to be predictive of the development of insulin resistance, the Insulin Resistance Syndrome and Type 2 diabetes.[8,9] Furthermore, cardiovascular morbidity and

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mortality is increased in patients with elevated CRP levels.[10] This adds to the evidence that chronic inflammation may be an underlying cause of both atherosclerosis and insulin resistance. hs-CRP has been widely studied and established as a marker which would reclassify patients into more accurate risk categories leading to more appropriate treatment decisions. Possibility of myocardial infarction occurring in setting of normal lipid levels is not uncommon. In an effort to better identify patients with high cardiovascular risk, several other biomarkers are being studied and hsCRP is one of the well studied ones. The recommendation suggests serum hs-CRP value below 1mg/l, 1-3 mg/l and >3mg/l to be associated with low, intermediate and high future cardiovascular risk.[11] Studies regarding this are scant in our population which made us to take up this study. We aimed to find the level of hs-CRP and its association with different parameters of lipid profile in our diabetic population. Unlike a prospective study, this cross sectional study would not be able to find causal relationships but will certainly lay down basis for further studies.

Materials and Methods

This study is a cross sectional study conducted in the department of biochemistry with collaboration of the department of internal medicine at B.P. Koirala Institute of Health Sciences, Dharan, Nepal for duration of one year. A total of 168 consecutive patients attending endocrinology OPD either diagnosed as Type 2 Diabetic as per American Diabetes Association guidelines or already taking treatment for Type 2 Diabetes have been enrolled in the study. Dyslipidemia has been defined as per the National Cholesterol Education Programme (NCEP) Adult Treatment Panel (ATP) III guidelines. The patients were included irrespective of duration of disease. Patients having severe anaemia, taking statins for dyslipidemia, having any metabolic instability, any type of cutaneous or systemic infection, any CVS or renal complications and not intending to take part in the study were excluded. The ethical clearance was taken from the institutional ethical review board and informed consent was taken from the participants. Blood samples were collected after a minimum of 8 hrs of fasting. 2 ml venous blood was collected into plain vial. Blood samples were allowed to clot and were centrifuged at 3000rpm for 5 minutes to separate the serum. Different parameters of lipid profile viz Total Cholesterol (TC), High density lipoprotein (HDL), Low density lipoprotein (LDL), Triglycerides (TG) and hsCRP were measured in serum. Analysis was done in fully automated closed system- Roche/Hitachi cobas c 311. Data were in entered in Microsoft Excel 2007 and analysed using SPSS.

Normally distributed data has been presented as mean and standard deviation and non parametric data as median and inter quartile range. Pearson correlation test has been used to find correlation between parametric data and spearman rho test for non parametric data. Comparison of means has been done by student's t test in parametric data with two groups. Comparison of median values has been done by Mann Whitney U test in non parametric data with two groups. P value less than 0.05 has been considered significant.

Results

This study is a cross-sectional study with an attempt to observe the role of hsCRP in stratifying CVS risk in Type 2 Diabetics. The correlation of inflammatory marker hsCRP has been found out with parameters of lipid profile viz. TC, HDL-C, LDL-C and TG. Out of 168 patients, there were 91 females and 77 males. They had different occupations, 30 of them were job holders in either government or private offices, 36 had their own business, 60 females were housewives, 14 were farmers and the rest 28 have been categorised into 'other' occupation. This includes occupations like daily waged labours, cobblers etc and those males who didn't work at all. Among 168 subjects, 20 were vegetarians and rest 148 consumed meat. Also, out of 168 subjects, 109 were hypertensive taking medications for the same, and rest 59 had normal blood pressure.

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The means±SD of age, TC, HDL-C, LDL-C and HDL/LDL ratio of patients were 52.2±11.9 years, 182.9±41.9 mg/dl, 41.6±8 mg/dl, 94.9±20 mg/dl, and 0.47±0.18 respectively. The medians of TG and hsCRP were 152.5 (109, 195) mg/dl and 1.9 (0.9, 2.8) mg/dl respectively. The differences in the levels of these parameters among male and female patients were calculated but were not found to be significant (Table 1).

Table 1 Biochemical parameters in males and females

	Male (77)	Female (91)	'p' value
AGE (years)	52.7±11.9	51.84±12.1	0.64a
TC (mg/dl)	176.3±42.4	188.4±41	0.06a
HDL (mg/dl)	40.9±9.2	42.1±6.9	0.32a
LDL (mg/dl)	95.1±19.8	94.7±20.3	0.9a
HDL/LDL	0.4±0.1	0.5±0.1	0.8a
TG (mg/dl)	130 (99, 179)	167 (124, 217)	0.05b
hs-CRP (mg/dl)	1.6 (1.0, 2.8)	2.0 (0.9, 2.7)	0.33b

a Independent t test   bMan whitney U test

Dyslipidemia has been classified according to NCEP ATP III guidelines and we found out that hypercholestrolemia, hypertriglyceridemia and increased LDL-C was seen in 36%, 50% and 33% of subjects respectively. Level of HDL-C was low in 47% of females and 16% of males. We divided patients into three and two different groups on the basis of hs-CRP level and LDL level respectively. hs-CRP level 1mg/L, 1-3mg/L and >3mg/L has been classified as low, average and high risk groups for CVS events by American Heart Association. LDL has been established as an independent marker for CVS events and has been suggested to be kept below 100mg/dl in Type 2 Diabetes Mellitus. Although only 33% of patients were categorised as having increased CVS risk according to high LDL levels, altogether 75% of patients had increased CVS risk according to hsCRP levels where 57% had moderate and 18% had high CVS risk. (Table 2).

Table 2 Distribution of LDL among three groups of hs-CRP

DL(mg/dl)	hsCRP (mg/L)		
	<1(n=42)	1-3(n=96)	>3(n=30)
<100	38	75	0
≥100	4	21	30

Shows distribution of LDL among three groups of hsCRP. hs-CRP was found to have significant positive correlation with TC (r=0.286, <0.01), LDL (r=0.652, <0.01) and TG (r=0.299, <0.01) and significant negative correlation with HDL (r= -0.614, <0.01) and HDL/LDL ratio (r= -0.646, <0.01). It was found that values of TC, LDL, and TG were significantly higher in patients having >3mg/L of hs-CRP than those having hs-CRP 1-3 mg/L, which was in turn higher than those having hs-CRP <1mg/L. Also the levels of HDL and HDL/LDL ratio was significantly lowest in subjects having >3mg/L hs-CRP and highest in subjects having hs-CRP <1mg/L. (Table 3) elaborates values of parameters of lipid profile among 3 groups according to hsCRP.

Table 3 Comparison of different parameters of lipid profile among three groups of hs-CRP.

hs-CRP \ (mg/L)	<1	1-3	>3	'p' value
TCa (mg/dl)	154±35	191±39	196±41	<0.01*
HDLa (mg/dl)	47±7	43±6	33±5	<0.01*
LDLa (mg/dl)	83±12	92±20	114±14	<0.01*
HDL/LDLa	0.6±0.2	0.5±0.2	0.3±0.1	<0.01*
Triglyceridsb (mg/dl)	105 (99,158)	158 (121,127)	195 (135,263)	<0.01*

aANNOVA, bKruskal-Wallis test. * Significant at the level of p=0.01.

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Discussion

Dyslipidemia is a well-recognized CVS risk factor among diabetics. The typical diabetic dyslipidemia consists of high total cholesterol, triglycerides, LDL-C and low HDL-C. Among these, the LDL-C has been considered to be most atherogenic and hence requires strict control. But, Although LDL cholesterol still remains a highly contributary risk factor for cardiovascular disease, at least one-third of coronary events occur in individuals with LDL levels < 130 mg/dl, which is generally considered an average level in individuals without overt coronary artery disease.[12] One study[13] even quoted "Half of all myocardial infarctions occur in persons in whom plasma lipid levels are normal". This caused the scientific world to look into several other markers which would be able to improve detection of subclinical atherosclerosis. Some of such markers are lipid parameters like lipoprotein (a), apolipoprotein (apo) A-ı and Apo B-100; inflammatory biomarkers like C reactive protein and fibrinogen and nutritional biomarkers like total plasma homocysteine.

A prospective study compared CVS risk predicting capabilities of[12] such markers and concluded that hsCRP level was most powerful predictor in univariate analysis.[14] Our study showed that among the lipid parameters, the commonest one to be raised was serum Triglyceride level followed by Total cholesterol and then LDL levels suggesting hypertriglyceridemia to be the commonest lipid abnormality in the diabetics of our population as well. Females showed higher value of TG than males. The median hsCRP value was 1.9 mg/L, which indicates moderate CVS risk. Females had higher hsCRP level than males. This finding is in accordance with a study done by Graziella et al15 involving 3249 Type 2 Diabetic patients. They also found out that with respect to people with CRP values in the lowest tertile (<1.6 mg/L), those with CRP values in the highest tertile (> 4.4mg/L) had significantly higher values of TC, LDL, TG and significantly lower values of HDL. These finding match exactly with the results seen in our study and the differences are statistically highly significant.

Our study shows significant positive correlation between hs-CRP and parameters of lipid profile and significant negative correlation between hs-CRP and HDL. These findings are exactly in agreement with findings of a comparative study done by Palvasha et al.[16] They have found the similar correlations of hs-CRP and ferritin (marker of inflammation) with parameters of lipid profile. Similar findings are shown by studies done by Sung et al.[17] and Rhee at al.[18] CRP is a strong predictor for CVS events and according to Ridker et al, it is stronger predictor of heart attack and stroke than LDL cholesterol.[19] A study was aimed to find out relationships between the LDL cholesterol and CRP levels achieved after treatment with statins and the risk of recurrent myocardial infarction or death from coronary causes among 3745 patients with acute coronary syndromes. They found out that patients who had low CRP levels after statin therapy had better clinical outcomes than those with higher CRP levels, regardless of the resultant level of LDL cholesterol and hence suggested that strategies to lower cardiovascular risk with statins should include monitoring both CRP as well as cholesterol.[20] LDL has been established as an independent marker of CVS risk and its serum level has been suggested to be kept below 100mg/dl in Type 2 Diabetes. In our study there were 75 patients, who had normal level of LDL, <100mg/dl but hs-CRP was in the range of moderate CVS risk, i.e. 1-3mg/L (Table 2). It would be beneficial to be more cautious with these kinds of patients and start the recommended interventions to reduce CVS risk.

Low-grade inflammation plays an important role not only in the pathogenesis of Diabetes mellitus but also has an association with dyslipidemia in the diabetics. Elevated CRP levels have been associated with obesity, dyslipidaemia and hypertension, and are found in insulin-resistant patients with Type 2 diabetes.[21, 22] Many evidences support the causal role of CRP in CVS diseases. There is convincing experimental evidence linking C-reactive protein to plaque disruption and the onset of cardiovascular events. C-reactive protein mRNA and protein has been found to be abundantly present in

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atherosclerotic lesions.[23] The interventions known to reduce CVS risk e.g diet, exercise, cessation of smoking, and controlling blood pressure, also decrease hs-CRP levels.[24] CRP has been found to be stronger predictor of heart attack and stroke than LDL and also persons having high CRP and Low LDL have a higher CVS risk than those having low CRP and high LDL.[19] With all these knowledge in mind, if we measure levels of hs-CRP routinely in the patients of Diabetes, we will be able predict and delay CVS complications especially in those patients who have normal level of LDL and are considered to have low CVS risk.

Conclusion
Though estimation of hs-CRP is not performed routinely in patients of Type 2 Diabetes, all these studies and results from our study encourage to evaluate its level alongside lipid profile so that we can predict CVS complications earlier and better, and intervene accordingly to prevent them.

List of abbreviations
1. hsCRP : High sensitivity C reactive protein.
2. NCEP ATP III : National Cholesterol Education Programme Adult Treatment Panel.
3. CVS : Cardiovascular system.
4. TC : Total cholesterol.
5. HDL : High density lipoprotein.
6. LDL : Low density lipoprotein.
7. TG : Triglycerides.

Ethics approval and consent to participate
This study is a cross sectional study conducted in the department of biochemistry with collaboration of the department of internal medicine at B.P. Koirala Institute of Health Sciences, Dharan, Nepal for duration of one year. The ethical clearance was taken from the institutional ethical review board and informed consent was taken from the participants prior to conducting the study.

Consent for publication
Not applicable
Competing interests
None

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