Summary

Chronic non-communicable diseases are a major threat to global economy. Among the most common non-communicable diseases that afflict humans worldwide are coronary artery disease (CAD) and diabetes. CAD is the major cause of morbidity and mortality in patients with type 2 diabetes mellitus (T2DM). Diabetic patients have two to three times higher risk of CAD than that among subjects without diabetes. Traditional risk factors like diabetes, obesity, hypertension, dyslipidemia, age, physical inactivity, alcohol consumption, smoking and family history are insufficient to account for the excess of CAD in patients with diabetes. This has lead to the need for identification of newer risk factors. Of these, inflammatory risk factors are being considered to be the most important factors because inflammation participates in atherosclerosis from its inception and development to its ultimate endpoint, thrombotic complications. Although inflammation plays an important role in atherosclerosis, there are very few reports on the role of inflammatory risk factors in CAD, especially among diabetic patients.

Monocyte chemoattractant protein-1 (MCP-1), a CC chemokine, is involved in recruitment of monocytes to the arterial wall. This is one of the major steps in the inflammatory cascade leading to atherosclerosis. Some studies have reported elevated MCP-1 to be associated with CAD while others have reported a lack of such association. Pyruvate kinase type M2 (M2-PK) is a glycolytic enzyme which catalyzes the dephosphorylation of phosphoenolpyruvate to pyruvate. M2-PK occurs in dimeric and tetrameric forms. dimeric pyruvate kinase M2 (dM2-PK) is elevated in conditions with high rate of proliferation like malignancy and inflammation. It was hypothesized in the present study that plasma MCP-1 and dM2-PK might act as inflammatory risk factors for CAD in patients with type 2 diabetes.

Prospective studies on Western population have defined the traditional risk factors for CAD but their results cannot be generalized since association of risk factors with CAD differs in various populations. With an increase in the prevalence of diabetes in India, the prevalence of CAD associated with diabetes is also increasing, but it is
unclear how the risk of CAD is distributed among the diabetic patients. There are very few studies on the traditional and inflammatory risk factors for CAD in T2DM patients from India. To the best of my knowledge, there is no reported study on risk factors for CAD among T2DM patients from Punjab, especially Amritsar.

The objectives of the present study were to assess various traditional risk factors and two newer risk factors (MCP-1 and dM2-PK) for CAD among T2DM patients. The present case-control study was done in Amritsar on a total of 300 subjects including 100 (50 males and 50 females) T2DM patients with CAD, 100 (50 males and 50 females) T2DM patients without CAD, 50 (25 males and 25 females) CAD patients without any history of diabetes and 50 (25 males and 25 females) age and sex matched healthy subjects (without any history of diabetes and CAD). Individuals on anti-inflammatory drug therapy, with a history of cancer, renal disease, thyroid disorder, rheumatoid arthritis and acute infections were excluded from the study sample.

A proforma-based interview was used to collect information about habitat, sociodemographic variables, physical activity, smoking and alcohol consumption history. For determining obesity and body composition, anthropometric measurements i.e. height, weight, waist circumference, hip circumference, skinfolds (biceps, triceps, subscapular, suprailiac) were taken on each subject using the standard technique. Body composition was also determined using bioelectric impedance analysis (BIA). Blood pressure of each subject was measured using standard auscultatory method. 5 ml of fasting blood sample was collected from each subject. Lipid profile (cholesterol, triglyceride, HDL-cholesterol, LDL-cholesterol, VLDL-cholesterol) was estimated from serum using automated blood analyzer. MCP-1 and dM2-PK level was estimated from plasma by sandwich ELISA using the standard technique. Statistical Package for Social Sciences 16.0 (SPSS 16.0, SPSS Inc, Chicago III) was used for the statistical analysis of data.

It was evident from the results of the present study that mean age of diabetic patients with CAD was significantly higher than those without CAD (59.48±8.03 vs 51.39±10.36 years, p=0.000). However, there was no significant difference in the mean age of non-diabetic subjects with CAD and without CAD. The results of univariate
regression analysis in diabetic patients showed that age was significantly associated with CAD. Multivariate logistic regression analysis in diabetic patients also showed that age (p=0.001, odds ratio (OR)=1.090, 95% CI: 1.038-1.145) was an independent risk factor for CAD. It was evident from the present study that the risk of CAD among T2DM patients increased with an increase in the age.

It was evident from the present study that diabetic patients with CAD had significantly higher mean duration of diabetes as compared to diabetic patients without CAD (12.30±7.81 years vs 5.91±4.22 years, p=0.000). The results of univariate and multivariate regression analysis revealed that duration of diabetes was an independent risk factor for CAD in diabetic patients.

In the present study, diabetic patients with CAD had significantly higher mean value of systolic blood pressure (SBP) than those without CAD. However, no significant difference was observed in the mean value of diastolic blood pressure (DBP) among the two groups. Mean SBP and DBP of non-diabetic subjects with CAD were significantly higher than that of non-diabetic subjects without CAD. In univariate regression analysis, SBP was significantly associated with CAD while DBP was not significantly associated with CAD in diabetic patients. In multivariate logistic regression analysis, elevated SBP (p=0.031, OR=1.064; 95% CI: 1.006-1.126) was an independent risk factor for CAD in female diabetic patients but not in male diabetic patients. Among non-diabetic subjects, SBP (p=0.001, OR=1.277; 95% CI: 1.099-1.484) showed a strong association with CAD while DBP was not a significant risk factor among non-diabetic subjects in both sexes. It could be concluded from the present study that SBP was an independent risk factor for CAD in female diabetic patients as well as non-diabetic subjects. This might reflect the greater negative impact of diabetes on cardiovascular risk factors in women than men.

Obesity has been reported to be a significant risk factor for CAD in the general population but its association with CAD among diabetic patients is unclear. Body mass index (BMI), waist-hip ratio (WHR), waist circumference (WC) and percent body fat were used to assess obesity in the present study. There was no significant difference in
the mean value of BMI, WC and WHR among male and female diabetic patients with and without CAD. Male and female diabetic patients with CAD had significantly higher mean value of percent body fat determined by BIA as compared to those without CAD. However there was no significant difference in the mean value of percent body fat determined from skinfolds among diabetic patients (both sexes) with CAD as compared to those without CAD. On the other hand, male and female non-diabetic subjects with CAD had significantly higher mean value of BMI, WC, WHR and percent body fat (determined by BIA and skinfolds) than those without CAD.

In univariate regression analysis, percent body fat estimated by BIA was a significant risk factor while BMI, WC, WHR and percent body fat calculated from skinfolds were not found to be significant risk factors for CAD among male and female diabetic patients. However percent body fat was not associated with CAD in multivariate logistic regression analysis in diabetic patients. The results of multivariate logistic regression analysis in non-diabetic subjects revealed a significant association of obesity variables (BMI, WC, WHR, percent body fat estimated by BIA and skinfolds) with CAD. Thus the above mentioned obesity variables were not independent risk factors for CAD in male and female T2DM patients, but all these obesity variables showed significant association with CAD in non-diabetic subjects. It was concluded that the association of obesity variables with CAD in T2DM patients was not similar to that observed in non-diabetic subjects.

In the present study, although the prevalence of obesity was high, but there was no significant difference in the frequency of obesity between diabetic patients with CAD and without CAD. On the other hand, significant difference was observed in the frequency of obesity between non-diabetic subjects with and without CAD.

The findings of the present study showed that mean value of serum total cholesterol and LDL-cholesterol was significantly higher in diabetic patients with CAD than in those without CAD. Serum HDL-cholesterol was significantly lower in diabetic patients with CAD than those without CAD. No significant difference was observed in mean values of serum triglyceride and VLDL-cholesterol in diabetic patients with CAD.
and without CAD. Similar results were observed in non-diabetic subjects. But in multivariate regression analysis, only reduced HDL-cholesterol (p=0.001, OR=0.685; 95% CI=0.580-0.810) was an independent risk factor for CAD in T2DM patients. Among the non-diabetic subjects, elevated cholesterol and LDL-cholesterol and low HDL-cholesterol were independent risk factor for CAD. Lipid abnormalities were highly prevalent among diabetic and non-diabetic subjects in the present study. Diabetic subjects were more prone to lipid abnormalities (hypercholesterolemia, hypertriglyceridemia and low HDL-cholesterol) as compared to non-diabetic subjects. The high frequency of dyslipidemia in the subjects of the present study might be attributed to the intake of diet rich in saturated fats.

CAD has a multifactorial etiology with many of the risk factors being influenced by lifestyle. In the present study, the prevalence of smoking was low among the male subjects. Smoking was not a significant risk factor for CAD in diabetic as well as non-diabetic males. The lack of association of CAD with smoking in the present study could be attributed to low prevalence of smoking in Punjab or the under-reporting of smoking due to cultural and other barriers.

In the present study, alcohol consumption was an independent risk factor for CAD in multivariate analysis among diabetic males (p=0.004, OR=3.192; 95% CI: 1.434-7.101) as well as non-diabetic males (p=0.003, OR=2.271; 95% CI: 2.067-3.021). The association of smoking and alcohol with CAD among females could not be assessed in the present study since none of the females reported a history of smoking or alcohol consumption. Most of the subjects in the present study were sedentary in lifestyle and belonged to middle socioeconomic class. Physical activity and socioeconomic status were not significant risk factors for CAD in diabetic patients and non-diabetic subjects. It was also apparent from the present study that family history of CAD did not play a significant role in the development of CAD among diabetic patients.

Results of the present study showed that older age, longer duration of diabetes, reduced HDL-cholesterol and elevated plasma MCP-1 (both sexes), alcohol consumption (only in males) and elevated SBP (only in females) were the risk factors for CAD in
diabetic patients. Other than these factors, BMI, WC, WHR, percent body fat, cholesterol and LDL-cholesterol were also the risk factors for CAD in the non-diabetic subjects but not in diabetic patients. Thus the present study revealed that risk factors for development of CAD among diabetic subjects were not similar to those in non-diabetic subjects, implying a need for establishment of appropriate guidelines for identifying T2DM patients at risk of developing CAD. Diabetic patients with higher age and longer diabetes duration should be regularly screened for CAD. Control of dyslipidemia, hypertension and alcohol consumption might help in reducing the risk of CAD among diabetic patients.

Mean plasma MCP-1 concentration was significantly higher in diabetic patients with CAD than those without CAD (318.52±103.25 vs 214.61±66.02 pg/ml respectively, p=0.000). Similar results were observed when both the sexes were compared separately. Non-diabetic subjects with CAD also had significantly higher mean plasma MCP-1 level in comparison with those without CAD (216.19±64.66 vs 100.16±27.23 pg/ml, p=0.000). Plasma MCP-1 was found to be an independent risk factor for CAD in diabetic patients after multivariate logistic regression analysis (p=0.001, OR=1.010; 95% CI: 1.005-1.015). Plasma MCP-1 was also a significant risk factor when male and female diabetic patients were analyzed separately. In non-diabetic subjects also, plasma MCP-1 was an independent risk factor for CAD.

In Pearson correlation analysis, plasma MCP-1 showed a significant correlation with age, duration of diabetes, SBP, BMI, WC, WHR, percent body fat, cholesterol, LDL-cholesterol, HDL-cholesterol and physical activity in both sexes and also with alcohol consumption in males. Duration of diabetes, SBP, percent body fat, cholesterol and LDL-cholesterol were independently associated with plasma MCP-1 concentration in both sexes.

The sensitivity of MCP-1 as a marker for CAD was 96% and specificity was 40%, using cut-off level of 100 pg/ml. The results of the present study suggested that MCP-1 might prove useful as a marker for CAD and using other markers in combination
with MCP-1 could serve as a useful tool for the assessment of CAD, especially in diabetic patients.

Mean plasma dM2-PK (37.63±23.87 U/ml) was higher in diabetic patients with CAD as compared to those without CAD (33.32±21.07 U/ml), but there was no significant difference in plasma dM2-PK concentration among diabetic patients with and without CAD. In non-diabetic subjects, mean concentration of dM2-PK was significantly higher among non-diabetic subjects with CAD (21.23±10.83 U/ml) as compared to those without CAD (13.08±9.06 U/ml). In univariate regression analysis, plasma dM2-PK was not found to be a significant risk factor for CAD in T2DM patients and non-diabetic subjects. Plasma dM2-PK did not show any significant correlation in Pearson’s analysis with any of the CAD risk factors. The present study showed that plasma dM2-PK could not act as a marker for CAD.

The present study was the first comprehensive report from North India addressing the role of plasma MCP-1 and dM2-PK as inflammatory markers for CAD among T2DM patients. Larger prospective studies on these inflammatory markers are further needed to confirm the findings of the present study. It can be concluded from the present study that plasma MCP-1 and some traditional risk factors (age, duration of diabetes, SBP, HDL-cholesterol, alcohol consumption) can be used for identification of T2DM patients at risk for developing CAD. Hence the key to combating the increasing incidence of CAD and diabetes in Punjab is the control of traditional risk factors, along with the use of newer risk markers as screening tools. Initiation of population based studies to clarify the role of risk factors in the Indian context would help to institute effective preventive and control measures.