DISCUSSION

Non-communicable diseases have been recognized as a major cause of morbidity and mortality globally, especially in the South East Asia region. India has also been in a phase of transition which has lead to a shift in the disease spectrum from communicable to non-communicable diseases, particularly coronary artery disease (CAD) and type 2 diabetes mellitus (T2DM). According to World Health Report (2002), cardiovascular diseases (CVDs) would be the largest cause of death and disability in India by 2020. It was predicted that 2.6 million Indians would die due to CAD in 2020, constituting 54.1% of all CVD deaths. Agarwal et al. (2009) also reported that Indians, as a population, are prone to CAD. Similarly diabetes has also assumed epidemic proportions in India. Shaw et al. (2010) estimated the prevalence of diabetes among adults to be 6.4% in 2010, affecting 285 million adults.

Grundy et al. (1999) reported that 65 - 80% of the deaths from type 2 diabetes were due to cardiovascular or cerebrovascular complications. CAD has been reported as the major cause of mortality and morbidity in patients with T2DM. According to NCEP III (2001), traditional risk factors such as hypertension, atherogenic dyslipidemia, glucose intolerance and abdominal obesity played an important role in initiating and accelerating the complex process of atherosclerosis. Grundy et al. (2005) reported that constellation of these interrelated risk factors of metabolic origin directly promoted the development of CAD and T2DM. However these traditional risk factors have been unable to fully explain the increased risk of CAD in diabetic patients.

Inflammation has been reported to play a central role in the pathophysiology of atherosclerosis, starting from initiation, through progression, and ultimately the thrombotic complications of atherosclerosis. Inflammatory factors are being considered the most important newer risk factors for CAD and it has been proposed that the understanding of the concept of inflammation in diabetes-accelerated atherosclerosis might be used to predict future cardiovascular risk. Despite the alarming burden of CAD in patients with T2DM in India, there is lack of proper guidelines on CAD risk factors among T2DM patients. Hence, in the present study, an attempt has been made to study
the various traditional risk factors (age, duration of diabetes, hypertension, obesity, lipid profile, family history of CAD and lifestyle related factors) and newer risk factors i.e. monocyte chemoattractant protein-1 (MCP-1) and dimeric pyruvate kinase M2 (dM2-PK) for CAD in T2DM patients.

The results of the present study have been discussed under the following headings:

5.1 Traditional risk factors for coronary artery disease

5.2 Inflammatory risk factors for coronary artery disease

5.3 Implications and future prospects

5.1 Traditional risk factors for coronary artery disease

Various epidemiological studies have identified several risk factors for CAD. The traditional risk factors for CAD assessed in the present study were age, duration of diabetes, hypertension, obesity, dyslipidemia, lifestyle related factors and family history of CAD.

5.1.1 Age

Ageing has been reported to have an important association with cardiovascular disease. Costopoulos et al. (2008) reported that atherosclerosis was a disease of ageing and the prevalence of CAD increased with an increase in age.

It is evident from Table 12 that the 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). Similarly, when male and female diabetic patients were compared separately, patients with CAD were significantly older as compared to those without CAD (Table 13). However, there was no significant difference in the mean age of non-diabetic subjects with CAD and without CAD (Table 14). The results of univariate regression analysis in diabetic patients showed that age was significantly (p=0.000) associated with CAD (Table 32). Multivariate logistic regression analysis in diabetic patients showed that age [p=0.001, odds ratio (OR)=1.090, 95% confidence interval (CI): 1.038-1.145] was an
independent risk factor for CAD (Table 22). Similar results were observed when both the sexes were compared separately (Table 34 and 35).

Some previous studies from India have also reported a similar association of age with CAD. Agrawal et al. (2004) conducted a clinic based study on T2DM patients (N=4400, mean age= 50.7±12.4 years) and reported a strong association of age with CAD (OR=2.16). In the Chennai Urban Population Study (CUPS) on native urban South Indian population, Mohan et al. (2001) reported that in multivariate logistic regression analysis, age had a significant (p<0.001, OR=1.05) association with CAD in diabetic patients. In a clinic based study from Patna, Achari et al. (2006) studied the risk factors for CAD in patients with T2DM and reported that age was significantly (p=0.000, OR=1.06; 95% CI: 1.03-1.09) associated with CAD. Idris et al. (2008), in a cross-sectional analysis of diabetic patients from the Chennai Urban Rural Epidemiological Studies (CURES), reported that age was a strong independent predictor of CAD risk. In another study from South India, Ramachandran et al. (1999) reported that age was significant risk factor for CAD. In a recent clinic based study from North-west India, Agarwal et al. (2009) reported that diabetic patients diagnosed with silent CAD had significantly higher mean age as compared to those without CAD.

Studies from other countries have also shown age to be significant risk factor for CAD in T2DM patients. Tseng (2003) studied CAD risk factors among Chinese T2DM patients and observed that patients with CAD had significantly higher mean age (63.6±0.7 vs 59.4±0.5 years, p<0.001) as compared to those without CAD and in multivariate logistic regression analysis, age (p=0.005, OR: 1.05; 95% CI: 1.02-1.07) was an independent risk factor for CAD. Ezenwaka and Offiah (2001) reported that age had significant impact on the cardiovascular risk in obese and non-obese patients with T2DM in the West Indies. Nelson et al. (1990), in a study on Pima Indians from the Gila River Indian Community in Arizona, reported that the rate of fatal CAD among the diabetic subjects increased with advancing age. Koistinen et al. (1994) showed that age of the patient along with duration of diabetes were the only factors associated with asymptomatic CAD in diabetic patients. Lunetta et al. (1997), in a study on patients with T2DM ranging in age from 35 to 70 years, reported that age increased the risk of CAD in
both men and women. Bo et al. (1999) studied risk factors for CAD in normal weight and overweight or obese patients with T2DM (N=2113) and reported that age was a significant risk factor for CAD in obese females with T2DM. Daghash et al. (2007), in a study on Qatari patients with T2DM, reported that there was statistically significant difference in age between diabetic patients with CAD and without CAD. Klein (1995) and Varghese et al. (2001) also found that age was the single most important time related variable for macrovascular disease.

However, age showed a lack of association with CAD among T2DM patients in some studies. Tsai et al. (2004) and Yoo et al. (2009) reported a lack of independent association of age with CAD in diabetic patients.

It was evident from the present study that the risk of CAD among T2DM patients increased with an increase in the age.

5.1.2 Duration of diabetes

Diabetes has been associated with an increased risk of CAD. In the present study, diabetic patients with CAD had significantly longer 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) as shown in Table 12. Duration of diabetes was significantly associated with CAD in univariate (p=0.001) as well as multivariate regression analysis (p=0.002, OR=1.129; 95% CI: 1.046-1.218) as evident from Table 32 and 33. Similar results were observed when both the sexes were compared separately (Table 34 and 35). In other words, duration of diabetes was an independent risk factor for CAD in diabetic patients.

Similar results were observed in some previous studies from North and South India. Agarwal et al. (2009) reported that diabetic patients diagnosed with silent CAD had significantly higher duration of diabetes as compared to those without CAD and suggested that T2DM patients with a mean duration of 10 years or above should undergo carotid Doppler examination to detect silent CAD. Mohan et al. (1995) reported that the prevalence of CAD increased with increase in duration of diabetes and approximately 40% of the subjects with more than 20 years of diabetes history had CAD. In another
study on T2DM patients with mean duration of diabetes 8.3±4.7 years, Agrawal et al. (2004) reported that duration of diabetes was an independent risk factor for CAD.

Studies from western countries have also reported duration of diabetes as significant risk factor for CAD in T2DM patients. Yoo et al. (2009) reported that in multivariate regression analysis, duration of diabetes ≥10 years was independent risk factor (OR=3.38; 95% CI=1.29-8.84) for CAD in T2DM patients. Lunetta et al. (1997), in a study on patients with T2DM with duration of diabetes of 1 to 30 years, reported that longer disease history increased the risk of CAD in both men and women. Nelson et al. (1990) reported that the rate of fatal CAD among the diabetic subjects increased with increasing duration of diabetes. In a study on normal weight and obese patients with T2DM, Bo et al. (1999) reported that duration of diabetes was a significant risk factor for CAD in obese males with T2DM. Ezenwaka and Offiah (2001) reported that duration of diabetes had significant impact on the cardiovascular risk.

However, some studies showed that duration of diabetes was not a significant risk factor for CAD among T2DM patients. Tsai et al. (2004) reported that duration of diabetes was not significant risk factor for silent CAD in multiple logistic regression analysis. Tseng (2003) reported that although duration of diabetes was significantly higher (10.7±0.6 vs 8.5±0.4 years, p<0.005) in diabetic patients with CAD as compared to those without CAD, it was not an independent risk factor for CAD. Ramachandran et al. (1999) and Achari et al. (2006) also reported a lack of association of CAD with duration of diabetes.

It could be concluded from the present study that the risk of developing CAD among T2DM patients increased with an increase in the duration of diabetes.

5.1.3 Hypertension

Hypertension has been associated with an increased incidence of CVD mortality. Elevated blood pressure has been reported to be an important public health problem in India. Rodgers et al. (2000) reported that hypertension was directly responsible for 57%
of all stroke deaths and 24% of all CAD deaths in India. According to the World Health Report (2002), hypertension was a major risk factor for stroke, cardiac failure and CAD.

In the present study, diabetic patients with CAD had significantly higher mean value of systolic blood pressure (SBP) than those without CAD (140.35±13.97 mm Hg vs 134.00±14.77 mm Hg, p=0.002) as shown in Table 12. However, no significant difference was observed in the mean value of diastolic blood pressure (DBP) among diabetic patients with CAD and without CAD. Similarly male and female diabetic patients with CAD had significantly higher SBP than those without CAD (Table 13). Mean SBP and DBP (Table 14) of non-diabetic subjects with CAD were significantly higher than that of non-diabetic subjects without CAD (146.06±16.12 mm Hg vs 126.92±13.11 mm Hg, p=0.000 respectively for SBP; 87.54±7.16 vs 82.00±6.70 mm Hg, p=0.000 respectively for DBP). In univariate regression analysis, SBP was significantly (p=0.001) associated with CAD while DBP was not significantly associated with CAD in diabetic patients (Table 32). 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 (Table 34 and 35). In univariate analysis among non-diabetic subjects, SBP (p=0.000) and DBP (p=0.01) showed a significant association with CAD (Table 36). In multivariate analysis, 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 (Table 37). Hence the present study showed that SBP was an independent risk factor for CAD in diabetic patients (females) as well as non-diabetic subjects.

The significant association of hypertension with CAD observed in the present study was in accordance with earlier studies. Agrawal et al. (2004) reported that mean SBP and DBP of diabetic patients were 138.3±14.8 mm Hg and 85.7±6.2 mm Hg respectively. SBP (OR=22; 95% CI: 15.89-30.45) and DBP (OR=7.50; 95% CI: 5.66-9.92) were independent risk factors for CAD in logistic regression analysis. Agarwal et al. (2009) reported that SBP was higher in diabetic patients with silent CAD as compared to those without CAD, while there was no significant difference in DBP. Hypertension (OR=2.0) was reported to be significantly associated with CAD. Achari et al. (2006)
reported that mean SBP of T2DM patients with CAD was significantly higher than those without CAD (142.6±21.3 mm Hg vs 130.2±19.1 mm Hg, p<0.036) and hypertension was a significant (OR=1.53; 95% CI: 1.03-2.28) risk factor for CAD.

Hypertension was also found as an independent predictor of CAD in T2DM patients in many prospective studies from Western countries (Walters et al. 1994, Schrier et al. 2007). A review of the impact of traditional risk factors on the occurrence of CVD in T2DM with meta-analyses by Orchard (1996) showed cholesterol, blood pressure and smoking to have significant role in development of CVD in T2DM. In the Diabetes Intervention Study, Hanefeld et al. (1997) showed that SBP was an independent risk factor for myocardial infarction in the 11-year follow up of T2DM patients. In the United Kingdom Prospective Diabetes Study (UKPDS), Turner et al. (1998) showed that raised SBP was a major risk factor for CAD in patients with T2DM, with a 15% increased risk for an increase in SBP of 10 mm Hg, which was similar to that reported in the general population by MacMahon et al. (1990). In a study assessing the relation between SBP and the risk of complications in patients with T2DM (N=4801), Adler et al. (2000) reported that each 10 mm Hg decrease in mean SBP was associated with 12% reduction in risk for any complication related to diabetes, 15% for deaths related to diabetes and 11% for myocardial infarction. This study further proved the significant contribution of SBP to cardiovascular risk in T2DM patients. In another study, Arauz-Pacheco et al. (2004) reported that people with both diabetes and hypertension had approximately twice the risk of CVD as compared to non-diabetic people with hypertension. Tseng (2003) reported that hypertension was an independent risk factor for CAD (p<0.05, OR=1.56; 95% CI: 1.01-2.41) among T2DM patients.

The present study showed that SBP was a significant risk factor for CAD in diabetic females but not in diabetic males (Table 34 and 35). Although no explanation for the sex difference in SBP as a risk factor for CAD in the present study was readily apparent, it might reflect the greater negative impact of diabetes on cardiovascular risk factors in women than men. In a follow up study on type 2 diabetic and non-diabetic subjects, Juutilainen et al. (2004) reported that contribution of elevated blood pressure to diabetes related CAD risk was greater in women as compared to men. Howard et al.
(1998) also reported gender differences with more adverse effects of diabetes on blood pressure in women as compared to men.

The significant association of hypertension (assessed by SBP and DBP) with CAD among non-diabetic subjects in the present study was similar to that reported in previous studies. In a case-control study of acute myocardial infarction in 52 countries, representing every inhabited continent (INTERHEART study), Yusuf et al. (2004) reported that history of hypertension (OR 1.91) was significantly related to acute myocardial infarction worldwide in both sexes and at all ages in all regions. In another study on cardiovascular risk factors among obese and non-obese patients with CAD, Yologlu et al. (2005) showed that hypertension was the strongest predictor for CAD irrespective of BMI. In a study on urban population of Siliguri (India), Mandal et al. (2009) reported that 47.2% of the subjects had hypertension and both SBP and DBP were significantly associated with ischemic heart disease. Several studies from North India (Wander et al. 1994, Singh et al. 1997a, Singh et al. 1998a, Gupta et al. 2004) also reported that hypertension was a significant risk factor for CAD in general population.

However, in contrast to the present study, hypertension has not been reported as a significant risk factor for CAD among diabetic patients in some reports (Laakso et al. 1993, Kuusisto et al. 1994, Lehto et al. 1997, Yoo et al. 2009). Nelson et al. (1990) also reported that hypertension was not a significant predictor of CAD mortality among patients with T2DM. Bo et al. (1999) reported that SBP and DBP were not independent risk factors for CAD in diabetic patients, although the prevalence of hypertension was significantly higher in diabetic patients with CAD (50.8% in males and 71% in females) as compared to those without CAD (36.9% in males and 60.1% in females).

In the present study, there was a significant difference in the frequency of hypertension among diabetic patients with CAD (42%) and without CAD (17%) as evident from Table 20. Non-diabetic subjects with CAD also had a significantly higher frequency of hypertension (58%) than non-diabetic subjects without CAD (2%) as shown in Table 22.
The high prevalence of hypertension in the present study was similar to that reported in previous studies. In a study on urban and rural populations of North India, Singh et al. (1998a) reported that the prevalence of hypertension in diabetics was 51.9% in urban and 29.4% in rural as compared to non-diabetics who had 21.9% in urban and 16.9% in rural population. Ezenwaka and Offiah (2001) reported that 48% of T2DM patients had a DBP >83 mm Hg. Tseng (2003) reported that there was a significant difference in the prevalence of hypertension among diabetic patients with CAD (51.4%) and without CAD (32.9%). Rani et al. (2005) reported that the frequency of hypertension was 30% among patients with T2DM. Achari et al. (2006) reported that the prevalence of hypertension was 38.9% among diabetic patients. In a study on coronary risk factors in Jaipur (Jaipur Heart Watch Study-I) using a random sample of 2,212 adults, Gupta et al. (1995) reported that the prevalence of hypertension (≥140/90 mm Hg) was 31% (males 30%, females 34%). In a similar study conducted after 7 years (Jaipur Heart Watch Study-II), Gupta et al. (2002) reported that the prevalence of hypertension was 33.7% in the general population. On the basis of various epidemiological studies, Gupta (2004) reported that the prevalence of hypertension ranged between 20-40% in urban adults and 12-17% among rural adults in India.

The prevalence of hypertension among diabetic patients in the present study (Table 20) was lower as compared to some previous studies. Song and Hardisty (2008) reported that prevalence of hypertension was 77.7% among patients with T2DM. Scheffel et al. (2004) reported that the prevalence of hypertension was 73% in patients with T2DM. Agarwal et al. (2009) reported that the frequency of hypertension was 90.9% among diabetic patients with CAD and 88.89% among those without CAD. Yoo et al. (2009) observed that 71.1% of diabetic patients with CAD had hypertension, which was significantly (p<0.05) higher than those without CAD (43.4%). The frequency of hypertension in the present study was higher than that reported by Ramachandran et al. (1998) in a study from South India in which the prevalence of hypertension in diabetics was 29.3% and non-diabetics was 24.4%.

Hence it was evident from the present study that hypertension was a highly prevalent CAD risk factor among diabetic patients. The association of hypertension with CAD was significant in diabetic (females) as well as non diabetic subjects (both sexes).
5.1.4 Obesity

Obesity has been associated with a harmful effect on cardiovascular system and increased cardiovascular mortality in various epidemiological and clinical studies, as reported by Romero-Corral et al. (2006). A continual increase in the prevalence of obesity in India during the past few years has been reported (Ramachandran 2004, Wasir and Misra 2004). Obesity is involved in the development of both diabetes and CAD.

In the present study, various commonly used measures i.e. body mass index (BMI), waist-hip ratio (WHR), waist circumference (WC) and percent body fat were used to assess obesity. Male diabetic subjects with CAD had higher mean value of BMI than those without CAD (27.46±3.57 kg/m$^2$ vs 26.61±3.78 kg/m$^2$), but this observed difference was statistically non significant (Table 13). There was no significant difference in the mean value of WC and WHR among male diabetic patients with and without CAD. Male diabetic patients with CAD had significantly higher mean value of percent body fat determined by BIA as compared to those without CAD (32.28±4.87 vs 28.19±5.14, p=0.000). However there was no significant difference in the mean value of percent body fat determined from skinfolds among male diabetic patients with CAD as compared to those without CAD. On the other hand, male 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 (Table 15).

The results of univariate regression analysis in male diabetic patients showed that BMI, WC and WHR were not significantly associated with CAD (Table 32). Among diabetic males, percent body fat (estimated by B1A) was a significant risk factor for CAD in univariate regression analysis but not in multivariate logistic regression analysis (Table 32 and 34). On the contrary, BMI (p=0.021), WC (p=0.031), WHR (p=0.005), percent body fat estimated by BIA and skinfolds (p=0.003) were independent risk factors for CAD in multivariate logistic regression analysis among male non-diabetic subjects (Table 36 and 38).
As evident from Table 13, mean value of BMI in diabetic females with CAD (28.54±3.58 kg/m$^2$) was higher than that for diabetic females without CAD (27.35±4.41 kg/m$^2$), but the difference between the two groups was statistically non-significant. Mean value of percent body fat (determined by BIA) in diabetic females with CAD was significantly higher as compared to those without CAD (39.50±6.01 vs 37.06±5.86, p=0.042). There was no significant difference in the mean value of WC, WHR and percent body fat (determined from skinfolds) among female diabetic patients with and without CAD. On the contrary, mean value of BMI among female non-diabetic subjects with CAD was significantly higher than those without CAD (27.74±3.66 kg/m$^2$ vs 25.76±2.23 kg/m$^2$, p=0.025) as shown in Table 15. Similarly mean value of WC, WHR and percent body fat (determined by BIA and skinfolds) was significantly higher in female non-diabetic subjects with CAD 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 female diabetic patients as shown in Table 32. However percent body fat was not associated with CAD in multivariate logistic regression analysis in female diabetic patients (Table 37). The results of multivariate logistic regression analysis in female non-diabetic subjects revealed a significant association of obesity variables [BMI (p=0.03), WC (p=0.007), WHR (p=0.023), percent body fat estimated by BIA (p=0.04) and skinfolds (p=0.024)] with CAD (Table 39).

Hence the present study revealed that various obesity variables i.e. BMI, WC, WHR and percent body fat were not independent risk factors for CAD in male and female T2DM patients. However, all these obesity variables (BMI, WC, WHR and percent body fat) showed significant association with CAD in non-diabetic subjects. It could be concluded from the present study that the association of obesity variables with CAD in T2DM patients was not similar to that observed in non-diabetic subjects.

Several previous studies also showed that obesity variables were not significant risk factors for CAD among diabetic patients. Yoo et al. (2009) reported that there was
no significant difference in the mean BMI of diabetic patients with CAD and without CAD \((25.2 \pm 2.8 \, \text{kg/m}^2 \, \text{vs} \, 24.8 \pm 4.4 \, \text{kg/m}^2)\) and BMI was not a significant risk factor for CAD. In a study on Chinese patients with T2DM, Tsai \textit{et al.} (2004) reported that there was no significant difference in the mean BMI and WHR of diabetic patients with and without CAD and both these obesity variables were not significantly associated with CAD. In a clinic based study on subjects with normal glucose tolerance, impaired glucose tolerance and diabetes, Mohan \textit{et al.} (2001) reported that BMI was not a significant risk factor for CAD in multivariate regression analysis. Tseng (2003) reported that percent body fat was a better predictor for CAD than BMI and WHR in Chinese type 2 diabetic patients resident in Taiwan. Percent body fat was significantly associated with CAD while BMI and WHR were not associated with CAD in multivariate analysis. Arora \textit{et al.} (2007) reported that mean values of BMI, WHR and percent body fat were \(26.47 \pm 5.04 \, \text{kg/m}^2, 0.99 \pm 0.06\) and \(34.71 \pm 8.57\) respectively in T2DM patients from Amritsar. Anjana \textit{et al.} (2004) reported that BMI was not associated with visceral fat in diabetic patients, suggesting that BMI was not good in predicting visceral adiposity in diabetic patients. Song and Hardisty (2008) showed that diabetic patients possessed adverse cardiovascular risk factors irrespective of their baseline body weight and concluded that obesity did not play a significant role in CVD among diabetic patients. Ezenwaka and Offiah (2001) reported that there was no significant difference in the prevalence of CVD risk factors among obese and non-obese patients with T2DM.

The lack of relationship observed between obesity variables and CAD among diabetic patients in the present study sample was also consistent with earlier reports (Knowler \textit{et al.} 1991, Sprafka \textit{et al.} 1993). In Pima Indians with T2DM, Nelson \textit{et al.} (1990) reported that BMI was not strongly associated with death from cardiovascular disease. In a study on Finnish patients with newly diagnosed T2DM, Uusitupa \textit{et al.} (1993) showed that BMI, WHR and skinfold thickness did not predict mortality from CVD. Klein \textit{et al.} (1997) studied the relationship of obesity to the incidence of microvascular and macrovascular complications in patients with diabetes and reported that obesity was not associated with microvascular and macrovascular complications. In another study on T2DM patients, Lunetta \textit{et al.} (1997) reported that BMI and WHR did not have any significance in the occurrence of CAD for either sex. In the UKPDS, Turner
et al. (1998) reported that obesity was not a significant factor in the development of CVD in T2DM patients. Morricone et al. (1999) investigated the degree of CAD in relation to obesity and fat distribution and showed that CAD in diabetic obese patients was unrelated to BMI and parameters of fat distribution.

The significant association between obesity variables (BMI, WC, WHR, percent body fat) and CAD in non-diabetic subjects in the present study was similar to that reported in the previous studies. Ramachandran et al. (1998), in a study on urban south Indians, reported that central obesity was the most important cardiovascular risk factor. Singh et al. (1999) reported that subjects with high and moderate percent body fat were associated with high prevalence of CAD. In the Jaipur Heart Watch study, Gupta et al. (2002) found a high prevalence of obesity in urban men and women of Jaipur and reported that obesity was a significant risk factor for CAD. Lubree et al. (2002) studied cardiovascular risk factors in 149 rural, 142 slum dwellers and 150 urban middle class Indian men in relation to their body fat. All cardiovascular risk factors were strongly related to the percent body fat and WHR in their study, suggesting that obesity was a significant risk factor for CAD. In the INTERHEART study, Yusuf et al. (2004) reported that abdominal obesity was significantly related to acute myocardial infarction worldwide in both the sexes. Rossi et al. (2009) showed that BMI (p=0.045) was an independent predictor of acute coronary events in Cox regression model.

However, in contrast to present study, some studies reported a significant association between obesity variables and CAD in T2DM patients. Achari et al. (2006) found a significant difference in the prevalence of obesity among diabetic patients with and without CAD. Obesity was significantly associated with CAD in diabetic patients in multivariate logistic regression analysis. In a study on Taiwanese T2DM patients, Tseng (2006) reported that patients with BMI≥25 kg/m² and WC ≥90 cm (men) or ≥80 cm (women) had much higher risk of CAD when compared to those without either risk factor. In another study, Cho et al. (2008) reported that abdominal obesity was associated with atherosclerosis in T2DM patients. In a clinic based study from Delhi (North-west India), Agarwal et al. (2009) reported that central obesity was significantly associated with silent CAD in patients with T2DM.
In the present study, there was no significant difference in the frequency of obesity between diabetic patients with CAD and without CAD (Table 20) while there was a significant difference in the frequency of obesity between non-diabetic subjects with and without CAD (Table 22). The frequency of obesity assessed by BMI, WHR, WC, percent body fat (BIA) and skinfolds was slightly higher in diabetic patients with CAD (71%, 76%, 76%, 83% and 76% respectively) than diabetic patients without CAD (62%, 73%, 73%, 77%, 69% respectively) as shown in Table 20. The frequency of obesity on the basis of BMI, WHR, WC, percent body fat (BIA) and skinfolds was 58%, 66%, 56%, 78% and 80% respectively in non-diabetic subjects with CAD and 22%, 34%, 16%, 42% and 44% respectively in non-diabetic subjects without CAD (Table 22).

Abdominal obesity was seen in a high proportion of subjects in the present study. It was not only present in the subjects obese according to BMI, but also in subjects with normal BMI. Indeed the overall prevalence of abdominal obesity was higher than the prevalence of generalized obesity (assessed from BMI). These data suggested that while abdominal obesity frequently coexisted with generalized obesity, it could also be present in the absence of generalized obesity.

Similar to the results of the present study, a high prevalence of obesity in T2DM patients ranging from 50% to 92% has been reported in previous studies. Vikram et al. (2003), in a study on T2DM patients from northern India, reported a high prevalence of generalized as well as abdominal obesity in T2DM patients. According to this study, 53.9% of males and 88.6% of females had abdominal obesity while 73.2% of males and 92.2% of females had generalized obesity. In a clinic based study from United Kingdom, Daousi et al. (2006) reported that 86% of patients with T2DM were overweight or obese, 52% were obese and 8.1% had morbid obesity. Monteiro et al. (2007) reported that the prevalence of obesity was 67% in T2DM patients asymptomatic for CAD. Agarwal et al. (2009) reported that the prevalence of obesity among T2DM patients on the basis of BMI and WHR was 57.1% (males:54.9%, females: 61.5%) and 40.7% (males:50.9%, females:27.8%) respectively. However, Klein et al. (1997) had reported a low prevalence of obesity (25.2%) among T2DM patients in a population-based study performed in southern Wisconsin.
It could be concluded from the present study the there was an increased prevalence of obesity among diabetic patients as well as non-diabetic subjects. Although obesity variables (BMI, WC, WHR and percent body fat) were significantly associated with CAD in non-diabetic subjects, none of these variables were significant risk factor for CAD in diabetic patients. Hence, according to the results of the present study, obesity variables cannot be used to identify T2DM patients at high risk of CAD.

5.1.5 Lipid Profile

Dyslipidemia is an important constituent of metabolic syndrome. It is also considered to be one of the major contributing factor for CAD. T2DM has also been reported by Haffner (2003) and Krauss (2004) to be associated with cluster of plasma lipid and lipoprotein abnormalities. Changes occurring in diabetic dyslipidemia have been reported to be both quantitative and qualitative in nature. Quantitative changes include increase in low density lipoprotein (LDL) cholesterol levels and decrease in high density lipoprotein (HDL) cholesterol due to hepatic lipase activity and decrease in VLDL-cholesterol; and an increase in very low density lipoprotein (VLDL) cholesterol due to increased availability of glucose for VLDL-cholesterol synthesis. Qualitative changes include non enzymatic glycation of LDL-cholesterol and HDL-cholesterol, leading to increased risk of CAD (Dietchy 1997, Arora et al. 2007).

The findings of the present study showed that mean value of serum total cholesterol (Table 16) was significantly higher in diabetic patients with CAD than in those without CAD (216.37±29.29 vs 207.93±29.72 mg/dl, p=0.044). Serum HDL-cholesterol was significantly lower in diabetic patients with CAD than those without CAD (42.38±3.34 vs 44.33±3.42 mg/dl, p=0.000). LDL-cholesterol levels were significantly higher in diabetic patients with CAD as compared to those without CAD (133.99±26.50 vs 124.66±26.10 mg/dl respectively, p=0.013). No significant difference was observed in mean values of serum triglyceride and VLDL-cholesterol in diabetic patients with CAD and without CAD. Similarly, among the non-diabetic subjects (Table 18), mean serum cholesterol concentration was significantly higher in those with CAD than without CAD, but no significant difference was observed in mean values of serum
triglycerides and VLDL-cholesterol. Mean LDL-cholesterol level was significantly higher in non-diabetic subjects with CAD as compared to those without CAD (131.39±25.28 mg/dl vs 91.65±26.71 mg/dl, p=0.000). Mean HDL-cholesterol was significantly lower in non-diabetic subjects with CAD as compared to those without CAD.

The results of univariate regression analysis revealed that increased total cholesterol and LDL-cholesterol and reduced HDL-cholesterol were significantly associated with CAD in diabetic patients (Table 32). Among the lipoproteins, 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 in multivariate logistic regression (Table 33). Among the non-diabetic subjects, elevated cholesterol and LDL-cholesterol and low HDL-cholesterol were significantly associated with CAD in univariate as well as multivariate logistic regression analysis (Table 36 and 37).

It was evident from the present study that only reduced HDL-cholesterol was a significant risk factor for CAD in T2DM patients. Several prospective studies have also reported reduced HDL-cholesterol to be an independent risk factor for CAD in diabetic patients. In a study on Chinese T2DM patients, Tseng (2003) reported that there was a significant difference in mean cholesterol and HDL-cholesterol levels among T2DM patients with and without CAD and in multivariate logistic regression analysis, HDL-cholesterol was a significant risk factor for CAD. No significant difference was observed in mean triglyceride, LDL-cholesterol and VLDL-cholesterol in their study. The report by Laakso et al. (1993) from Finland was a comprehensive study of lipoproteins in relation to CAD in T2DM. In multiple logistic regression analysis, only low HDL-cholesterol predicted CAD in their study. Lunetta et al. (1997) investigated the role of lipoproteins in CAD among T2DM patients and reported that low HDL-cholesterol was independently related to CAD in both the sexes while cholesterol, LDL-cholesterol and VLDL-cholesterol were significant risk factors only in diabetic men. Goldschmid et al. (1994) reported that men with diabetes showed an approximately 30% increase in risk of CAD from highest to lowest tertile of HDL-cholesterol levels, while women with diabetes had almost an 8-fold increase in risk. Bo et al. (1999) showed that the
prevalence of dyslipidemia was significantly higher in diabetic females with CAD as compared to those without CAD (41.9% vs 29.2%) but after multiple logistic regression analysis, only reduced HDL-cholesterol was a predictor of CAD in females. No significant difference was observed in the frequency of dyslipidemia among diabetic males with CAD and without CAD (35.7% vs 35.5%). Lien *et al.* (1996) reported that low HDL-cholesterol was associated with increased risk of CAD even if triglyceride and total cholesterol levels were not elevated. Jacobs *et al.* (1990) showed that a 10 mg/dl decrease in HDL-cholesterol conferred the same risk for CAD as 30 mg/dl increase in LDL-cholesterol.

Some studies reported that in addition to HDL-cholesterol, other lipid abnormalities were also associated with CAD among T2DM patients. Kannel (1985), in the Framingham study, reported that triglyceride levels were higher and HDL-cholesterol levels were lower in diabetics when compared to non-diabetics. Turner *et al.* (1998), in the UKPDS, showed that elevated LDL-cholesterol and decreased HDL-cholesterol were major risk factors for CAD in diabetic subjects. In a study on T2DM patients from Bikaner (North west India), Agrawal *et al.* (2006) reported that high levels of VLDL-cholesterol, triglycerides, LDL-cholesterol and low HDL-cholesterol were significantly associated with CAD. Ramachandran *et al.* (2001a) showed the lipid profile of diabetic patients with CAD had a higher concentration of total cholesterol, triglyceride, LDL-cholesterol, LDL-cholesterol/HDL-cholesterol ratio and a lower concentration of HDL-cholesterol. Rajmohanan *et al.* (2000), in a large clinic-based study on 17,855 type 2 diabetic subjects, reported that the prevalence of CAD was significantly higher among patients with isolated hypercholesterolemia, isolated high LDL-cholesterol and isolated low cholesterol compared with normolipidemic individuals.

In contrast to the present study, some studies showed that dyslipidemia was not associated with CAD in T2DM patients. Achari *et al.* (2006) reported that total cholesterol, triglyceride, LDL-cholesterol, HDL-cholesterol and VLDL-cholesterol were not significant risk factors for CAD in diabetic patients. Similarly, Yoo *et al.* (2009) reported that none of the lipoproteins (total cholesterol, triglyceride, LDL-cholesterol,
HDL-cholesterol and VLDL-cholesterol) were significant risk factors for silent CAD in T2DM patients.

According to the results of the present study, cholesterol, LDL-cholesterol and triglycerides were not significantly associated with CAD in T2DM patients. But some previous studies have provided contrasting data. In the CUPS, Mohan et al. (2001) reported that serum cholesterol, LDL-cholesterol and total cholesterol/HDL-cholesterol ratio were elevated in subjects with CAD as compared to those without CAD. The prevalence of CAD significantly increased with increase in quartiles of total cholesterol and LDL-cholesterol levels. However, LDL-cholesterol had the strongest association with CAD on regression analysis. Abbott et al. (1988) reported that hypercholesterolemia was a risk factor for CAD in diabetic as well as non-diabetic population. In a study from North India, Singh et al. (2006) showed that diabetic patients with CAD had significantly higher levels of total cholesterol and triglycerides than non-diabetic patients with CAD. Clinical trials reported by Shepherd et al. (2006) and Decewicz et al. (2009) in diabetic and CAD patients showed that aggressive cholesterol management in patients with diabetes and CAD lead to significant reduction in cardiovascular events.

Similar to the present study, an increased concentration of LDL-cholesterol/total cholesterol and reduced HDL-cholesterol at baseline has been reported as a major risk factor for CAD among non-diabetic subjects in several studies (Law et al. 1994, Scandinavian Simvastatin Survival Study Group 1994, Shepherd et al. 1995, Anand et al. 2000, Singh et al. 2006). In a large retrospective study on 5748 CAD patients and 8103 healthy controls, Achari and Thakur (2004) reported that serum cholesterol, LDL-cholesterol and total cholesterol to HDL ratio was higher among the CAD subjects compared to normal subjects. Their study also reported that there was a lack of association of serum triglycerides levels with CAD. Burman et al. (2004) documented that LDL-cholesterol level and total cholesterol/HDL cholesterol ratio was higher in CAD patients compared to controls but there was no significant difference in serum triglyceride levels. In a case-control study from North India, Singh et al. (2006) reported that total cholesterol and HDL-cholesterol were significant predictors of CAD. Assmann and Schulte (1992) showed that HDL-cholesterol and triglyceride were significant CAD
risk factors in univariate analysis. In multivariate analysis, only HDL-cholesterol was found to be a significant risk factor. Krishnaswami et al. (1996), in a study from South India, showed that total cholesterol and triglyceride levels had equal expression in the development of atherosclerotic disease, with cholesterol being of greater relevance in those <50 years and triglyceride being of greater relevance in those > 50 years.

In the present study, hypercholesterolemia (>200mg/dl) and hypertriglyceridemia (>150mg/dl) were present in 60% and 67% of diabetic patients with CAD and 38% and 65% of those without CAD (Table 20). 45% of diabetic patients with CAD and 22% of those without CAD had low HDL-cholesterol (<40 mg/dl). The frequency of hypercholesterolemia, hypertriglyceridemia and low HDL-cholesterol was 52%, 44% and 34% respectively in non-diabetic subjects with CAD and 10%, 26% and 8% respectively among non-diabetic subjects without CAD (Table 22).

As shown by the above data, lipid abnormalities were highly prevalent among diabetic and non-diabetic subjects in the present study. Several previous studies have also reported a high prevalence of lipid abnormalities among T2DM patients. Agrawal et al. (2004), in a study from North-West India, determined the association of dyslipidemia with micro and macro vascular complications in T2DM. 15% of patients had high serum cholesterol (>240mg/dl), 42.4% had elevated serum triglycerides (>160mg/dl) and 52.3% of patients had low HDL-cholesterol levels. The study by Udawat et al. (2001) reported that incidence of dyslipidemia was higher in diabetic subjects than non-diabetic subjects and there was a high prevalence of dyslipidemia in diabetic patients. The authors reported dyslipidemia in 89% of diabetic patients, raised LDL-cholesterol (>100 mg%) in 6%, low HDL-cholesterol (<35 mg%) in 58% and hypertriglyceridemia (> 200 mg%) in 22% patients. Monteiro et al. (2007) reported that the prevalence of HDL-cholesterol <45 mg/dl was 69%, LDL-cholesterol ≥100 mg/dl was 85% and triglycerides ≥150 mg/dl was 54% among diabetic patients. Agarwal et al. (2009) showed that the prevalence of high cholesterol (>200mg%), high LDL (>140mg%), low HDL (<40mg%) and high triglycerides (>150mg%) was 40.9%, 27.3%, 45.5% and 59.1% respectively in diabetic patients with CAD and 29.6%, 9.3%, 38.9% and 55.6% respectively in those without CAD.
It could be concluded from the present study that 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. An important and unstudied aspect of the fat intake is the effect of various Punjabi cooking habits on fatty acid intake. Shallow frying, which is highly prevalent in Punjabi kitchens, can lead to oxidation of fatty acids and formation of cholesterol oxides which are toxic to arterial endothelium. Reduced HDL-cholesterol was a significant risk factor for CAD in T2DM patients while high cholesterol and LDL-cholesterol and low HDL-cholesterol were CAD risk factors among non-diabetic subjects.

5.1.6 Lifestyle related factors

5.1.6.1 Smoking

Cigarette and tobacco smoke is known to contain many toxic and vasoactive substances. The smoking habit has become an epidemic in many countries with the growth of cigarette manufacturing industry.

In the present study, no significant difference was observed in the frequency of smoking among diabetic males with and without CAD. The frequency of current smoking was low i.e. 14% in diabetic males with CAD and 10% in those without CAD (Table 21). Among non-diabetic males, there was a significant difference in the smoking status of those with and without CAD. 8% of diabetic males with CAD and 4% of those without CAD were current smokers (Table 23). Smoking was not a significant risk factor for CAD in diabetic as well as non-diabetic males in univariate regression analysis (Table 32 and 36). 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. Begom and Singh (1995) also observed that the prevalence of smoking in South Indian males (44.6%) and passive smoking in South Indian females (45.3%) was significantly higher than in North Indians.
Similar to the results of the present study, many previous studies also reported that smoking was not a significant risk factor for CAD in diabetic patients. Yoo et al. (2009) reported that there was no significant difference in the frequency of smoking among diabetic patients with CAD (42.1%) and without CAD (51.3%). Tseng (2007), in a study on Taiwanese T2DM patients, showed that smoking was not associated with peripheral arterial disease. Singh et al. (2006) reported that there was no significant difference in the frequency of smoking among diabetic patients with CAD (46%) and without CAD (61%). Patil et al. (2004) reported that smoking was not a significant risk factor in acute myocardial infarction in patients from rural parts of India.

However, in contrast to the results of the present study, smoking was reported as a significant risk factor for CAD among T2DM patients in some studies. Monteiro et al. (2007) reported that 46% of T2DM patients had a history of smoking and smoking was a significant risk factor for myocardial ischemia. Bo et al. (1999) reported that CAD among diabetic males was significantly associated with years of smoking. Turner et al. (1998) reported that smoking was positively associated with CAD among patients with T2DM.

5.1.6.2 Alcohol consumption

CAD has a multifactorial etiology with many of the risk factors being influenced by lifestyle. In the present study, significant difference (p=0.002) was observed in the frequency of alcohol consumption among diabetic males with and without CAD. 16% and 28% of diabetic males with CAD reported current and former consumption of alcohol respectively (Table 21). Among diabetic males without CAD, the frequency of current and former alcohol consumption was 6% and 6% respectively. Significant difference was observed in alcohol consumption among non-diabetic males with and without CAD (Table 23). 20% and 28% of non-diabetic males with CAD while 4% and 8% of those without CAD were current and former consumers of alcohol respectively. Among diabetic males, alcohol consumption showed a significant association with CAD in univariate as well as multivariate analysis (p=0.004, OR=3.192; 95% CI: 1.434-7.101) (Table 32 and 34) Similarly, alcohol consumption also showed a significant association
with CAD in multivariate regression analysis (p=0.003, OR=2.271; 95% CI: 2.067-3.021) among non-diabetic males (Table 38). The effect of alcohol consumption on the development of CAD among females in the present study could not be evaluated since none of the females reported consumption of alcohol.

Alcohol consumption was significantly associated with an increased risk of CAD among both diabetic and non-diabetic male subjects in the present study. Most of the males in the present study reported heavy alcohol consumption (>3 drinks per day) or binge drinking. Previous studies reported contrasting data about association of alcohol with CAD i.e. alcohol consumption was associated with an increased risk of CAD in some studies while others reported that alcohol consumption reduced the CAD risk. Alcohol consumption was reported by O’Keefe et al. (2007) to be associated with CAD risk and all-cause mortality in a J- or U-shaped manner. This showed that there was a reduced risk of CAD mortality among moderate consumers of alcohol as compared to abstainers and heavy drinkers. In the MONICA (Monitoring of Trends and Determinants in Cardiovascular Disease) project, Malyutina et al. (2002) reported that men who consumed >5 drinks per day had a 2-fold increased risk for acute myocardial infarction and all-cause mortality compared with those who did not drink at all. The authors further observed that men who consumed 1 or 2 drinks daily had a 50% reduction in risk of acute myocardial infarction compared with abstainers. Goldberg et al. (1994) reported a prospective study of the health effects of alcohol consumption in middle-aged and elderly men. Alcohol consumption was determined at baseline and 6 years later. After adjustment for confounding variables, the investigators reported that total mortality followed a J-shaped pattern in relationship to alcohol in men aged 51 to 75 years. Death from CAD was lowest for heavy drinkers among middle-aged men and lowest for moderate drinkers among elderly men. Rani et al. (2005) reported that the prevalence of alcohol consumption among diabetic males was 10.6%. In the INTERHEART study, Yusuf et al. (2004) reported that regular alcohol consumption was significantly related to reduced myocardial infarction worldwide in both sexes and at all ages in all regions. On the other hand, Bo et al. (1999) reported that alcohol consumption was not a significant risk factor for CAD in T2DM patients.
It could be concluded from the results of the present study that alcohol consumption significantly increased the risk of CAD in diabetic as well as non-diabetic males.

5.1.6.3 Physical activity

Physical inactivity has been reported as an important lifestyle factor in the increased susceptibility to CAD. It has an impact on many of the components of the metabolic syndrome. The rapid globalization and industrialization occurring in developing countries like India has produced much advancement on the social and economic front. These improved socio-economic conditions have also resulted in decrease in physical activity leading to an increase in T2DM and its related complications.

The question of how best to measure physical activity in epidemiological studies to ensure comparability between different populations has been debated many times, as reported by Wareham and Rennie (1998). Measurement of physical activity by questionnaire, although practical in epidemiological studies, generates measurement error which can weaken the observed relationship between the behavior and outcome of interest. In the absence of a standardized and validated approach to physical activity assessment, the questionnaire method was used in the present study.

In the present study, 82% and 78% of diabetic subjects with and without CAD respectively were doing low physical activity and there was no significant difference between the two groups (Table 20). Significant difference (Table 22) was observed in the physical activity level of non-diabetic subjects with CAD (low physical activity in 80%) and without CAD (low physical activity in 40%). In univariate regression analysis, physical activity was not a significant risk factor for CAD in diabetic patients and non-diabetic subjects (Table 32 and 36).

In the present study, most of the subjects were sedentary in lifestyle. Very few reports have assessed the association of physical activity with CAD among diabetic patients. Turner et al. (1998) reported that, in the UKPDS, physical activity was not a
significant risk factor for CAD in patients with T2DM. Bo et al. (1999) reported that there was significant difference in the physical activity of diabetic patients with CAD and without CAD but it was not a significant risk factor for CAD in multivariate logistic regression analysis.

In contrast to the present study, many studies on the general (non-diabetic) population showed that physical activity was a significant CAD risk factor. Rastogi et al. (2004), in a hospital-based case-control study from two urban centers in India, suggested that daily moderate intensity physical activity (the equivalent of briskly walking 35-40 min per day) was associated with a 55% lower risk for CAD. Gupta et al. (1995) showed that CAD was associated with sedentary habits in the Jaipur Heart Watch-2 Study. Hassan et al. (2005) studied the risk factors for CAD in rural areas of Peshawar (Pakistan) and reported that physical inactivity was significantly associated with the prevalence of CAD. Monteiro et al. (2007) reported that 64% of diabetic patients had sedentary physical activity.

Thus the present study revealed a lack of association of physical activity with CAD.

5.1.6.4 Socioeconomic Status

In developing countries like India, CAD has been thought to be predominantly a disease of the upper income groups. According to Cooper et al. (2000), as development progresses, this relation might reverse. In developed countries, CAD has been reported to be more prevalent among individuals with low socioeconomic status.

It was evident from Table 20 and 22 that most of the diabetic and non-diabetic subjects of the present study sample belonged to middle socioeconomic class. In univariate regression analysis, socioeconomic status was not found to be a significant risk factor for CAD in diabetic patients as well as non-diabetic subjects (Table 32 and 36).

Some studies on general population have revealed that subjects with higher socioeconomic status were at an increased risk of CAD. In a study from Delhi, Chadha et al. (1990) reported a lower prevalence of ECG abnormalities among people of a lower
socioeconomic status than among those of a higher status. Singh et al. (1998b) reported that social class 1, 2 and 3 (high and middle socioeconomic groups) in an urban population of North India had a higher prevalence of CAD and coronary risk factors i.e. hypercholesterolemia, hypertension, diabetes mellitus and sedentary lifestyle in both sexes. Gupta et al. (1994) showed that the risk of CAD showed a gradient with increase in affluence in urban and immigrant populations and from lower social classes to higher social classes. However some studies reported that individuals having lower socioeconomic status had higher risk of CAD. Singh et al. (2006) showed that socioeconomically deprived individuals were at higher risk of CAD than the better-off sections in Rajasthan. Steptoe et al. (2010) reported that education level was inversely associated with the risk of CAD.

It was apparent from the present study that socioeconomic status was not associated with CAD among diabetic as well as non-diabetic subjects.

5.1.7 Family history of CAD

Familial aggregation of CAD with a high prevalence among first degree relatives has been reported in several studies. In the present study (Table 20), although diabetic patients with CAD (27%) had a significantly higher frequency of a positive family history as compared to diabetic patients without CAD (12%). Non-diabetic subjects with CAD (Table 22) also had a significantly higher frequency of family history of CAD as compared to those without CAD (44% vs 10%). The results of univariate regression showed that family history was a significant risk factor for CAD in male diabetic patients but not in female diabetic patients (Table 32). Among non-diabetic subjects, family history was significantly associated with CAD in univariate regression analysis (Table 36). However, multivariate logistic regression analysis revealed that family history of CAD was not a significant risk factor for CAD in diabetic as well as non-diabetic subjects. The lack of association between the occurrence of CAD and family history in the present study might be because of ignorance in subjects about the disease in their family members or a reluctance to provide information of diseased individuals in the family because of social stigma.
Some previous studies on diabetic patients showed that family history was not a significant risk factor for CAD. Agarwal et al. (2009) reported that there was no significant difference in the family history of CAD among T2DM patients with and without silent CAD. Monteiro et al. (2007) reported that 16% of diabetic patients had family history of premature atherosclerotic disease while family history was not associated with myocardial ischemia in multivariate logistic regression analysis. In a study from North India, Singh et al. (2006) reported that there was no significant difference in the frequency of positive family history of CAD among diabetic patients with and without CAD.

However, in contrast to the present study, CAD was reported to be associated with family history among diabetic and non-diabetic subjects in many previous studies. Yoo et al. (2009) reported that family history was an independent predictor of CAD in diabetic patients with asymptomatic CAD. In a case-control study of CAD patients (N=200) and controls from Pakistan, Nishtar et al. (2004) reported that positive family history was an independent risk factor for CAD. Myers et al. (1990) reported that among men with low risk for CAD by risk factor profile (non-smoking, non-hypertensive persons), more than two thirds of those who experienced CAD, had a positive parental history. Wander et al. (1994) reported that positive family history was a significant CAD risk factor in an epidemiological study conducted in Pohir, situated near Ludhiana (Punjab).

It could be concluded from the present study that family history of CAD did not play a significant role in the development of CAD among diabetic patients.

### 5.2 Newer risk factors for coronary artery disease

Traditional risk factors for CAD cannot fully explain the increased risk of CAD among diabetic patients. In this context, several newer risk factors for CAD are being identified of which, inflammatory factors are of utmost importance. There is emerging evidence that inflammatory processes and immune mechanisms are involved in atherogenesis. MCP-1 and dM2-PK were the inflammatory factors assessed as risk factors for CAD in the current study.
5.2.1 Monocyte Chemoattractant Protein-1

5.2.1.1 Monocyte chemoattractant protein-1 as a risk factor for coronary artery disease

MCP-1 is a CC chemokine which plays an important role in inflammation, and hence atherosclerosis, by the recruitment of monocytes to the site of inflammation. As evident from Table 5, 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 (Table 17). 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) as shown in Table 18. In univariate regression analysis, plasma MCP-1 was significantly (p=0.000) associated with CAD (Table 32). 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) as shown in Table 33. Plasma MCP-1 was also a significant risk factor when male and female diabetic patients were analyzed separately (Table 34 and 35). In non-diabetic subjects, plasma MCP-1 showed a significant association with CAD in univariate regression analysis as well as multivariate regression analysis (p=0.004, OR=1.084; 95% CI: 1.026-1.146) as evident from Table 36 and 37.

MCP-1 has been assessed in some studies from the western population, addressing its influence in atherosclerosis or atherosclerosis-related diseases but its role has not been clear in these studies. Some studies reported a significant association of MCP-1 with CAD, similar to the present study. The study by Martinovic et al. (2005) was the first clinical report indicating elevated levels of plasma MCP-1 in patients at risk for CAD. Patients with either angiographically proven CAD or coronary risk factors showed significantly enhanced MCP-1 (125.7±10.6 pg/ml) serum levels in that study. Piemonti et al. (2003) reported that plasma MCP-1 concentration was associated with CVD mortality after a 7-year follow up period in middle-aged diabetic as well as non-diabetic individuals. Reynolds et al. (2003) demonstrated elevation of MCP-1 in a large group of stroke patients. In
the Atherosclerosis Risk in Communities study, Hoogeveen et al. (2005) determined the relationship between plasma MCP-1 level and peripheral arterial disease or CAD. Mean plasma MCP-1 levels were significantly higher in peripheral arterial disease or CAD cases (468.7 vs 416.5 pg/ml in non-cases). MCP-1 was significantly associated with peripheral arterial disease or CAD in multiple logistic regression analysis. Incident CAD risk increased significantly per standard deviation difference in MCP-1 level independent of other cardiovascular risk factors, including inflammatory markers. A polymorphism (2518G) in the MCP-1 promoter was demonstrated by Szalai et al. (2001) to be associated with an increased risk of CAD at the population level. Tang et al. (2007), in a population-based family study, reported that plasma levels of MCP-1 were associated with coronary artery calcification (a measure of the burden of coronary atherosclerosis). Piemonti et al. (2009) measured MCP-1 concentration in the plasma of 363 middle-aged overweight/obese individuals (15% with T2DM and 12% with impaired glucose tolerance) and reported that MCP-1 was increased in individuals with T2DM and showed significant correlation with biochemical risk markers of atherosclerosis. MCP-1 was also significantly associated with CVD mortality in that study. Kaur et al. (2009), in a study on South Indian Cohort, reported that MCP-1 -2518G allele might be contributing to CAD by conferring an increased risk to metabolic syndrome and/or obesity. Ohman et al. (2010) studied the role of MCP-1 on the progression of visceral fat-induced atherosclerosis and reported that MCP-1 deficient mice were resistant against atherosclerosis induced by transplanted visceral adipose tissue.

Elevated MCP-1 was also associated with other complications related to CAD in previous studies. Matsumori et al. (1997) reported that patients with acute myocardial infarction had higher levels of MCP-1 as compared to patients suffering from angina only. Aukrust et al. (1998) showed an elevation of MCP-1 expression in patients with acute coronary syndrome (ACS) and heart failure. Hokimoto et al. (2000) studied MCP-1 levels in patients after receiving coronary angioplasty and reported that the higher the plasma concentrations of MCP-1, the higher the likelihood of having a restenosis. Another study by Kitamoto et al. (2003) supported the hypothesis that circulating MCP-1 was a factor that contributed significantly to the development of atherosclerotic lesions. The authors reported that intramuscular transfection of an N-terminal deletion
mutant of MCP-1 gene suppressed monocyte infiltration/activation in the injured arterial wall and thus attenuated the development of arterial restenosis. Petrkova et al. (2004) demonstrated that MCP-1 was elevated in circulation of patients with peripheral arterial disease.

However, in contrast to the present study, some reports have indicated a lack of association of circulating MCP-1 with CAD. Mosedale et al. (2005) did not find any significant relationship of plasma MCP-1 concentration with atherosclerotic burden and the Framingham risk score. Van Mieghem et al. (2005) revealed that the concentrations of hs-C-reactive protein (hsCRP), interleukin-6 (IL-6) and MCP-1 were not associated with changes in novel invasive imaging modalities, such as palpography or virtual histology of the coronary arteries. Deepa et al. (2006), in a cross-sectional study from South India, reported that no significant difference was observed in serum MCP-1 levels among subjects with normal glucose tolerance, impaired glucose tolerance, insulin resistance and diabetes. Liang et al. (2006) reported that novel risk factors, such as concentrations of serum CD40L, MCP-1 and adhesion molecules, did not play significant roles in CAD progression.

It could be concluded from the present study that elevated MCP-1 was a significant risk factor for CAD in diabetic as well as non-diabetic subjects.

5.2.1.2 Association of monocyte chemoattractant protein-1 with traditional risk factors for coronary artery disease.

In the present study, the plasma concentration of MCP-1 showed a significant correlation with age in Pearson’s correlation analysis \(r=0.184, p=0.020\) as evident from Table 24. However, current age was not independently associated with plasma MCP-1 in multivariate linear regression analysis among both sexes (Table 26). Similar to the results of present study, some previous studies showed lack of an independent association of plasma MCP-1 with age. Petrkova et al. (2004) and Arakelyan et al. (2005) showed that age was not independently associated with MCP-1 concentration. In contrast to the present study, Inadera et al. (1999) and de Lemos et al. (2003) reported that MCP-1 was positively associated with age.
Plasma MCP-1 concentration also showed a significant correlation \((r=0.495, p=0.000)\) with duration of diabetes in the present study (Table 24) and was independently associated with duration of diabetes (Table 26). Chronic subclinical inflammation is an essential component of insulin resistance syndrome. Diabetes leads to the formation of advanced glycation end products (AGEs), which bind to the receptors of various cells like monocytes, leading to increased production of various inflammatory molecules like MCP-1 by these cells. As the duration of diabetes increases, AGEs accumulate, leading to increased production of MCP-1. Previous studies by Piemonti et al. (2003) and Deo et al. (2004) have also shown diabetes to be associated with increased MCP-1.

As evident from Table 24, SBP \((r=0.229, p=0.000)\) showed independent correlation with plasma MCP-1 levels. However no significant correlation of MCP-1 with DBP was observed. Multivariate linear regression analysis (both sexes combined) revealed that SBP \((p=0.028)\) was an independent predictor of plasma MCP-1 concentration (Table 26). Increasing evidence supports the view that inflammation might participate in hypertension providing a pathophysiological link between these two diseases. Angiotensin II (which is increased in hypertension) might be the physiological factor linking MCP-1 with hypertension. Angiotensin II has been reported to increase the expression of proinflammatory cytokines such as IL–6 and MCP-1 by arterial smooth muscle cells (Hernandez-Presa et al. 1997, Kranzhofer et al. 1999, Tummala et al. 1999).

Some previous studies also showed that MCP-1 level was significantly associated with hypertension. de Lemos et al. (2003) reported that MCP-1 was positively associated with hypertension. Larrouse et al. (2006) demonstrated that serum concentrations of MCP-1 were higher in salt-sensitive high blood pressure patients. Chobanian (1990) demonstrated that there was endothelial dysfunction (leading to increased levels of inflammatory molecules like MCP-1) in experimental hypertension, hence supporting the association of hypertension with MCP-1. In contrast, Arakelyan et al. (2005) investigated MCP-1 levels in serum by ELISA in healthy control subjects, patients with ischemic stroke, and in patients with myocardial infarction and reported that MCP-1 concentration was not associated with hypertension.
Obesity has been closely related with the over-expression of chemokines. In the present study, BMI (r=0.160, p=0.000), WC (r=0.146, p=0.011), WHR (r=0.319, p=0.000), percent body fat estimated by BIA (r=0.342, p=0.000) and percent body fat obtained from skinfolds (r=0.290, p=0.000) were significantly correlated with plasma MCP-1 in both sexes as shown in Table 24. Multivariate linear regression analysis (both sexes combined) revealed that WC (p=0.000) and percent body fat (p=0.003) were independently associated with plasma MCP-1 concentration while BMI and WHR were not independently associated with MCP-1 as shown in Table 26.

Adipocytes and macrophages participate in an intricate cellular network which is formed during the development of obesity and contributes to the pathophysiology of obesity-related complications like T2DM and atherosclerosis. Adipose tissue has been reported to be metabolically active and secrete various chemokines and proinflammatory cytokines such as MCP-1 and tumor necrosis factor-α (TNF-α). Production of these proteins by adipose tissue is increased in obesity leading to a positive association of MCP-1 with obesity (Mohamed-Ali et al. 1997, Sethi and Hotamisligil 1999).

Several studies have reported an association of MCP-1 with obesity. Xu et al. (2003) showed that obesity in mice was associated with significant upregulation of immune genes in adipose tissue including MCP-1. Malavazos et al. (2005) reported that obese patients had higher MCP-1 levels, suggesting that visceral fat predisposed to cardiac dysfunction through low grade inflammation. Kim et al. (2006) reported that the plasma concentration of MCP-1 was higher in obese subjects than in controls. Catalan et al. (2008) showed that MCP-1 expression levels were upregulated in obese normal glucose tolerant and obese T2DM patients compared to lean subjects. Chacon et al. (2007) reported that circulating MCP-1 was correlated with WHR but not with BMI. But some studies have reported contrasting results. Herder et al. (2006b) found no evidence that systemic MCP-1 concentrations were associated with parameters of obesity like BMI, WHR and body fat mass, indicating that the contribution of adipose tissue to circulating MCP-1 levels might be low. de Lemos et al. (2003)
reported that there was no association of MCP- with BMI in patients with acute coronary syndrome.

In present study, cholesterol \((r=0.187, \ p=0.002)\) and LDL-cholesterol level \((r=0.244, \ p=0.000)\) was positively correlated with the plasma MCP-1 level as shown in Table 24. Plasma MCP-1 was negatively associated with HDL-cholesterol \((r=-0.141, \ p=0.014)\) i.e. an increase in plasma MCP-1 concentration was associated with decrease in HDL-cholesterol concentration. Triglyceride and VLDL-cholesterol did not show any significant correlation with plasma MCP-1. In multivariate linear regression analysis, cholesterol \((p=0.002)\) and LDL-cholesterol \((p=0.049)\) showed independent association with plasma MCP-1 as evident from Table 26. The significant association of lipids with MCP-1 could be explained by the fact that LDL undergoes oxidative modification to form ox-LDL (Berliner et al. 1997, Williams and Tabas 1998). ox-LDL has been reported to be involved in the formation of MCP-1 in macrophages and endothelium (Quinn et al. 1988, Cushing et al. 1990). The negative correlation of HDL-cholesterol with plasma MCP-1 indicated the protective role of HDL-cholesterol in inflammation and atherosclerosis.

The correlation of MCP-1 with markers of lipid metabolism has also been reported in previous studies. In a study of patients with hypertensive disease, Parissis et al. (2000) studied the differences in serum activity of various inflammatory molecules (granulocyte-macrophage colony-stimulating factor, MCP-1 and macrophage inflammatory protein-1α) between hypertensive patients with and without significant hyperlipidemia before receiving any medical treatment. Serum activity of the studied inflammatory factors was more elevated in hypertensive patients with significant hyperlipidemia. Petrkova et al. (2004), in a study on peripheral artery disease, showed that MCP-1 was correlated with total cholesterol, LDL-cholesterol and triglycerides confirming that the relationship between the chemokine and lipids was a general feature of atherosclerosis irrespective of its form and/or localisation. Herder et al. (2006a) revealed a significant positive association between systemic MCP-1 and elevated triglyceride levels. Maeno et al. (2000) reported that lipoproteins isolated from diabetics stimulated the expression of MCP-1 m-RNA in cultured
endothelial cells. Dwivedi et al. (2001) showed that oxLDL increased MCP-1 mRNA levels and hence monocyte adhesion in an endothelial cell line. Han et al. (1999) reported that expression of both MCP-1 and its monocyte receptor were upregulated in hypercholesterolemia. Gu et al. (1998) showed that LDL receptor–deficient mice that underwent targeted deletion of the MCP-1 gene were resistant to the effects of a cholesterol-rich diet and had markedly less atherosclerosis than similar mice who did not have a deletion of the MCP-1 gene.

As evident from Table 25, plasma MCP-1 was significantly higher (p=0.017) in males who consumed alcohol as compared to those who did not consume alcohol. However, the difference in MCP-1 levels among the current, former and non smokers was statistically non significant (p=0.065). No significant independent association of MCP-1 with alcohol consumption was observed in multivariate linear regression analysis (Table 27). Previous studies have reported contradictory data on the correlation of MCP-1 with alcohol intake and smoking. In a study on patients with acute coronary syndrome, de Lemos et al. (2003) reported that smoking was not associated with plasma MCP-1 levels. In a study involving patients with ischemic stroke, myocardial infarction and healthy, control subjects Arakelyan et al. (2005) observed no relationship of MCP-1 level with smoking and alcohol consumption. Augustynska et al. (2009) assessed MCP-1 serum levels in alcohol dependent women and reported that concentration of MCP-1 was significantly higher in alcohol-dependent female group compared to healthy subjects (360.34 +/-273.95 vs 240.27+/-178.31 pg/ml; p=0.030). However, the results of the present study were in contradiction with some previous studies reporting that alcohol consumption reduced inflammation by inhibiting monocyte migration. Verma et al. (1993) and Szabo (1999) showed that alcohol suppressed the synthesis of pro-inflammatory cytokines and chemokines (such as TNF-α, IL-1α, IL-6, IL-8 and MCP-1) in alveolar macrophages and human blood monocytes, both in vivo and in vitro.

In the current study, subjects who having high physical activity had significantly lower (p=0.041) levels of plasma MCP-1 as compared to subjects with low and moderate physical activity (Table 25). However physical activity was not associated with plasma MCP-1 in multivariate analysis (Table 26). Adamopoulos et al. (2001) investigated the
effects of physical training on serum activity of inflammatory markers i.e. MCP-1, granulocyte-macrophage colony-stimulating factor, soluble intercellular adhesion molecule-1 (sICAM-1) and soluble vascular cell adhesion molecule-1 (sVCAM-1) in patients with chronic heart failure. Physical training produced a significant reduction in serum GM-CSF, MCP-1, sICAM-1 and sVCAM-1. Troseid et al. (2004) reported that exercise decreased systemic MCP-1 concentrations in subjects with the metabolic syndrome. The reduction in MCP-1 levels due to exercise might be attributable to a complex mechanism whereby weight loss and modest physical exercise lead to a general and systemic attenuation of low-grade inflammation leading to reduction of MCP-1 release from stromal-vascular cells (Bruun et al. 2005, Di Gregorio et al. 2005, Fain and Madan 2005). In contrast to the present study, Becker et al. (2005) reported that MCP-1 serum levels were elevated after strenuous physical exercise in patients at risk for CAD. Similarly, plasma MCP-1 did not show a significant association with family history of CAD in the present study (Table 25). Deo et al. (2004) showed that family history of CAD was not independently associated with plasma MCP-1. In contrast to the present study, Martinovic et al. (2005) reported that MCP-1 levels were elevated in subjects with a family history of CAD.

5.2.1.3 Monocyte chemoattractant protein-1 as a marker for coronary artery disease

MCP-1 was also assessed as a marker for CAD in the present study. For this purpose, sensitivity and specificity of MCP-1 as a CAD marker was studied. Sensitivity is the ability to give positive results in true cases. Specificity is the ability to give negative results where the disease is absent. The cut-off values for MCP-1 among healthy individuals have not been established as yet. Previous studies have reported varying value of plasma MCP-1 among the control (healthy) subjects ranging from 74 pg/ml to 157 pg/ml (de Lemos et al. 2003, Piemonti et al. 2003, Martinovic et al. 2005, Berrahmoune et al. 2006). Hence the mean value of MCP-1 observed among healthy subjects in the present study was used as the cut-off for assessing the specificity and sensitivity of MCP-1 as a CAD marker.
In the present study, using a cut-off of 100 pg/ml, MCP-1 had a high sensitivity of 96% as a marker for CAD but the specificity was only 40% (Table 31). High sensitivity of MCP-1 implied that MCP-1 could correctly diagnose a high proportion of patients with CAD. On the other hand, low specificity of MCP-1 reflected low precision of MCP-1 to identify non-CAD patients i.e. some non-CAD individuals might show false positive results with MCP-1. The low specificity of MCP-1 in the present study might be due to the fact that diabetes leads to elevated level of inflammation, leading to elevated MCP-1 levels in some diabetic patients without CAD. So the present study showed that MCP-1 might be a better marker for CAD among non-diabetic subjects as compared to diabetic patients.

Some previous studies also assessed the role of MCP-1 as a biomarker for atherosclerosis. Kervinen et al. (2004) reported that MCP-1 could predict coronary events in patients with overt CAD during follow-up of 13 months. The increased plasma levels of MCP-1 were helpful for predicting new coronary events independent of other inflammatory mediators i.e. C-reactive protein (CRP) and IL-6. In a clinic based study, Martinovic et al. (2005) suggested that MCP-1 serum levels could serve as a direct marker of inflammatory activity in patients at risk for CAD. MCP-1 was also studied in patients after receiving coronary angioplasty (Hokimoto et al. 2000, Cipllone et al. 2001) and the findings indicated that the higher the plasma concentration of MCP-1, the higher the likelihood of having restenosis, highlighting the potential of using plasma MCP-1 as a biomarker to assess the prognosis of the acute coronary artery syndromes. de Lemos et al. (2003) also showed that MCP-1 could act as a biomarker in patients with acute coronary syndromes. A number of preventive interventions have been shown to reduce either tissue expression or circulating levels of MCP-1, including treatment with statins, thiazolidinediones, hormone replacement and consumption of red wine (Ghanim et al. 2001, Martinez-Gonzalez et al. 2001, Stork et al. 2002). These observations also suggested that MCP-1 might prove to be useful as a biomarker in patients with or at risk for atherosclerosis and its complication.
However, some studies suggested that MCP-1 was not useful as a marker for CAD. Coll et al. (2007) reviewed the role of MCP-1 as a biomarker for atherosclerosis and reported that although most of the studies linked MCP-1 to atherosclerosis, the relationship was not independent of the classical risk factors. They suggested that no suitable analytical tools were available to reach strong conclusions with respect to the value of MCP-1 concentration as a biomarker of atherosclerosis and recommended that the MCP-1/CCL2 pathway should be further explored as a diagnostic and prognostic target.

Multiple prospective epidemiological studies have shown that increased levels of cytokines (IL-1, IL-6, TNF-α), cell adhesion molecules (VCAM-1, P-selectin, E-selectin) and acute phase reactants (CRP, fibrinogen, serum amyloid-A) were associated with increased cardiovascular risk (Ridker et al. 2000a, Ridker et al. 2000b, Ridker et al. 2000c, Ridker et al. 2002). Using MCP-1 in combination with these markers might be a more useful tool for identifying individuals (especially diabetic patients) at increased risk of CAD. Ardigo et al. (2007) measured serum levels of seven chemokines [CXCL10 (IP-10), CCL11 (eotaxin), CCL3 (MIP-1 α), CCL2 (MCP-1), CCL8 (MCP-2), CCL7 (MCP-3), and CCL13 (MCP-4)] in 48 subjects with clinically significant CAD and 44 controls and concluded that a combination of serum levels of multiple chemokines identified subjects with clinically significant atherosclerotic heart disease with a very high degree of accuracy. Based on the participation of several chemokines in atherogenesis, Aukrust et al. (2008) suggested that combined measurements of multiple chemokines could act as a ‘signature of disease’ which might serve as a highly accurate method to assess for the presence of CAD. Models using multiple chemokines more accurately distinguished cases and controls compared with models using traditional risk factors.

It could be concluded from the present study that MCP-1 might act as a marker for CAD in diabetic as well as non-diabetic subjects. In non-diabetic subjects, MCP-1 might act alone as a marker for CAD. But, for diabetic patients, using other inflammatory markers in combination with MCP-1 could improve its specificity and make it a better tool for the assessment of CAD among these patients. However, these findings require conformation in further studies.
5.2.2 dimeric Pyruvate kinase M2

dM2-PK is the dimeric form of the glycolytic enzyme pyruvate kinase M2 which is present mainly in the cells having a high rate of proliferation like tumor cells and inflammatory cells. As evident from Table 16, 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 (p=0.177) in plasma dM2-PK concentration among diabetic patients with and without CAD. When male and female diabetic patients were compared separately, no significant difference was observed in mean dM2-PK concentration among those with CAD and without CAD (Table 17). In non-diabetic subjects, mean concentration of dM2-PK was significantly higher (p=0.000) among non-diabetic subjects with CAD (21.23±10.83 U/ml) as compared to those without CAD (13.08±9.06 U/ml) as shown in Table 18. In univariate regression analysis, dM2-PK was not found to be a significant risk factor for CAD in T2DM patients (Table 32). In univariate logistic regression analysis among non-diabetic subjects, dM2-PK was significantly (p=0.005) associated with CAD (Table 36). However, in multivariate logistic regression analysis, dM2-PK was not a significant CAD risk factor among non-diabetic subjects (Table 37).

In Pearson correlation analysis, none of the traditional risk factors i.e. age, duration of diabetes, SBP, DBP, BMI, WHR, percent body fat, cholesterol, triglyceride, HDL-cholesterol, LDL-cholesterol, VLDL-cholesterol, smoking, alcohol consumption and physical activity were significantly correlated with plasma dM2-PK concentration as shown in Table 29 and 30. Plasma dM2-PK also did not show any significant correlation with plasma MCP-1 concentration.

As evident from literature, there is no previous study analyzing dM2-PK as a risk factor for CAD. However, previous studies have reported elevated levels of dM2-PK in various other inflammatory conditions. McDowell et al. (2004) reported that chronic cardiac failure resulted in increased circulating plasma concentration of dM2-PK. Oremek et al. (2003b) showed that plasma level of dM2-PK was elevated in patients with type 1 diabetes and T2DM suffering from diabetic nephropathy. No significant
correlation was found between dM2-PK and metabolic markers such as HbA1c, glucose and the other investigated parameters. Oremek et al. (2003a) reported that EDTA-plasma concentration of dM2-PK was increased in patients with rheumatic diseases. Chung-Faye et al. (2007) reported that dM2-PK was significantly elevated in patients with intestinal inflammation and suggested that it could be a potential non invasive marker of disease activity in inflammatory bowel disease. Johnson et al. (2009) demonstrated that faecal dM2-PK was a sensitive marker of pouch inflammation and its concentration directly correlated with objective markers of pouchitis severity. Elevated dM2-PK levels were reported by Shastri et al. (2006) in about 88% of patients with inflammatory bowel disease. 67% of patients with an acute inflammatory reaction were found by Staib et al. (2006) to have elevated levels of dM2-PK using a cut-off level of 15 U/ml.

In the present study, dM2-PK was analyzed as a marker for CAD using the cut-off of 15 U/ml reported in literature for healthy individuals (Staib et al. 2006, Hardt and Ewald 2008). dM2-PK as found to have a low sensitivity (56%) and low specificity (33.3%), implying that it was not useful as a marker for CAD (Table 31). The present study was the first report assessing dM2-PK as an inflammatory marker for CAD, though dM2-PK has been reported to be a marker for various kinds of tumors in several studies reviewed by Hardt and Ewald (2008). However, since some of the diabetic and CAD patients in the present study had raised dM2-PK level than healthy individuals, it implied that patients with T2DM and/or CAD, when screened for malignancy using dM2-PK as a marker, might give false positive results.

From the literature, there is conflicting data on pyruvate kinase (PK) in diabetes. Studies on transcriptomic and proteomic profiles have revealed decreased PK activity and mRNA expression in adipose tissue, cultured pancreaetic islets of Type 1 diabetic patients and in animal models of diabetes (Belfiore et al. 1975, Kondoh et al. 1992, Lupi et al. 2004). Iori et al. (2008) also reported a reduction in pyruvate kinase activity in fibroblasts from diabetic patients with nephropathy. Insulin has also been reported to activate PK, leading to reduction in PK activity in insulin resistant states like T2DM. Cantley et al. (2010) have reported the use of inhibitors of M2-PK for clinical trials against diseases like obesity, diabetes and proliferation dependent diseases. However some studies also reported slightly increased or unchanged activity of PK in diabetic
patients (Diamant and Shafrir 1978, Rossi et al. 1990). Since the dimeric form of M2-PK has a low activity, the reduction in PK activity (as reported in the above studies) would signify an increase in dM2-PK in the tissue of diabetic patients. However, no significant increase was observed in the dM2-PK concentration in the plasma of diabetic patients in the present study. It might be possible that decreased PK activity (i.e. increased plasma dM2-PK) in the tissue in various studies might not be observed in the plasma of diabetic patients.

It could be concluded from the present study that dM2-PK was not a significant risk factor for CAD in diabetic and non-diabetic subjects. Further studies would be required to clarify the role of dM2-PK in diabetes and CAD.

5.3 Implications and future prospects

The prevalence and role of various risk factors for diabetes and CAD has been shown to have regional as well as ethnic disparity. The database to support treatment recommendations has been derived primarily from studies in Western countries. Therefore, the risk calculation using these data would underestimate or overestimate the risk in Asian Indians. The incomplete detection, treatment, and control of CAD risk factors has been a cause of concern to developing countries such as India. Studies on native Indians are required for the management of CAD among T2DM patients in India. Hence the present study was highly significant in this respect. There are ethnic differences along with variation in dietary habits and lifestyle in different regions of India. Most of the previous reports from India on CAD risk factors among diabetic patients were from South India. There were very few reports from North India, especially Punjab. To the best of my knowledge, the present study provided the first report on CAD risk factors in T2DM patients from Amritsar (Punjab).

In the current study, older age, longer duration of diabetes, reduced HDL-cholesterol, alcohol consumption and elevated plasma MCP-1 were the risk factors for CAD in male T2DM patients. In female T2DM patients, older age, longer duration of diabetes, increased SBP, reduced HDL-cholesterol and elevated plasma MCP-1 were the risk factors for CAD. BMI, WC, WHR, percent body fat, cholesterol and LDL-
cholesterol increased the risk of CAD in the non-diabetic subjects but not in diabetic patients. Thus the present study revealed that risk factors for development of CAD were not similar among diabetic and non-diabetic subjects. This implies that appropriate guidelines for identifying T2DM patients at risk of developing CAD should be established. The identification of risk factors for CAD specific to T2DM patients would provide means for decreasing CAD risk in them through more accurate determination of overall risk status, the reduction of modifiable risk factors and better treatment decisions.

It was apparent from the present study that risk of CAD among diabetic patients increased with increasing age and duration of diabetes, implying that diabetic patients with higher age and longer duration of diabetes should be regularly screened for CAD. Idris et al. (2008) reported that the transition from low to moderate risk category for men and women with diabetes occurred at ages 37 and 50 years, respectively and recommended that statins should be routinely prescribed to all Asian Indian men and women above the age of 37 and 50 years, respectively. Yoo et al. (2009) recommended routine screening of CAD in patients with diabetes duration ≥10 years.

Lipid abnormalities were highly prevalent among diabetic patients in the present study, emphasizing that control of dyslipidemia might help in reducing the risk of CAD among diabetic patients in this region. Various studies (Sacks et al. 1996, Pyorala et al. 1997, Goldberg et al. 1998) reported that the features of dyslipidemia in diabetics could be improved by physical activity and the use of statins, fibrates and nicotinic acid. Manchanda et al. (2000) reported that yoga lifestyle intervention program had favorable effects on angina as well as risk factors of CAD (body weight, lipid levels etc). Hence control of dyslipidemia might also be suggested as an additional measure for prevention of CAD.

The present study also implied that steps should be taken to control blood pressure in T2DM patients, especially women in order to reduce the risk of CAD. The prevalence of obesity was high in the present study, although obesity was not found to be a significant CAD risk factor among T2DM patients. Since obesity was not a significant risk factor for CAD in T2DM patients, it implied that the commonly used guidelines for
control of obesity to reduce CAD risk would not be applicable to these patients. The discordant relationship between obesity and CAD in diabetes, as observed in the present study and previous studies also raised the question on how obesity should be defined in T2DM. In this context, the concept of ‘metabolically obese normal weight’ (in which individuals despite having normal BMI demonstrate metabolic disturbances typical of obese individuals) has been emphasized previously in some studies (Ruderman et al. 1998, Katsuki et al. 2004).

Alcohol consumption was a significant and highly prevalent risk factor for CAD in males. This risk factor is modifiable and can be corrected by changing the lifestyle. Steps should be taken to reduce alcohol consumption by educating people especially diabetic patients about the harmful effects of alcohol.

Among the newer risk factors, MCP-1 was found to be a significant risk factor for CAD in T2DM patients in the present study. The present study was the first study from North India addressing the role of plasma MCP-1 in CAD among T2DM patients. There were only two previous reports (Deepa et al. 2006, Kaur et al. 2009) on circulating MCP-1 levels from India and both of these were from South India. When evaluated as a marker for CAD, MCP-1 was found to have a high sensitivity (96%) in the present study. However, the specificity of MCP-1 was found to be low (using 100 pg/ml as the cut-off). This implied that MCP-1 could identify almost all patients with CAD (high sensitivity); but some patients without CAD might also have elevated plasma MCP-1 (i.e. low specificity). Aukrust et al. (2008) suggested that a biomarker for CVD should reflect important pathophysiological processes in atherosclerosis, should provide independent information on CVD risk, should be easy to measure using inexpensive and standardized commercial assays with low variability. In this regard, MCP-1 proved to be a good marker. MCP-1 has been reported to have a central pathogenic role in atherosclerosis. The association of MCP-1 with CAD was also found to be independent of the traditional risk factors in the present study. Quantitative sandwich ELISA (using the kit from R&D Systems Inc. USA; Cat No. DCP00) was employed for the quantitative measurement of MCP-1 in EDTA-plasma. The test was not very expensive (costing approximately 800 Indian rupees). This kit has
been previously used in several studies (Becker et al. 2005, Martinovic et al. 2005, Deepa et al. 2006) for the determination of plasma MCP-1 levels with low variability (intra- and interassay coefficient of variation, 4.0% and 7.6%, respectively) and good reproducibility. Hence the results of the present study suggested that MCP-1 might prove useful as a marker for CAD. Since the specificity of MCP-1 was low, using other markers in combination with MCP-1 could serve as a useful tool to assess the presence of CAD, especially in diabetic patients. Larger prospective studies are needed to confirm the findings of the present study.


dM2-PK was not found to be significant marker for CAD in the present study. Diabetic and CAD patients in the present study had elevated levels of dM2-PK as compared to controls. Thus the presence of diabetes and CAD in the patients might give false positive data while using dM2-PK as a tumor marker. Previous studies also showed that dM2-PK was elevated in patients with non-malignant diseases (especially patients with acute inflammatory reaction). When interpreting the test results of dM2-PK as a tumor marker, these facts should always be kept in mind.

Substantial reduction in the incidence of CAD and diabetes can be achieved by primary prevention which is possible by creating awareness regarding risk factors among the populations. So there is an urgent need to develop CVD risk factor surveillance system in India. Suitable CAD prevention efforts should be initiated at population level in India.

CAD is a multifactorial condition for which diabetes is an important risk factor. A continuous increase in epidemic of diabetes in India has resulted in a larger population at risk for CAD and its related morbidity and mortality. However there is paucity of data on CAD risk factors among diabetic patients from India. Moreover, the high risk of CAD among Indians cannot be fully explained by traditional risk factors. There are very few studies on newer (especially inflammatory) risk factors on Indian diabetic patients.

The observations of the present study provide an insight into the role of traditional and newer risk factors in the emerging epidemic of CAD and diabetes. From the results of the present study, it can be inferred that increasing age, longer duration of diabetes,
Reduced HDL-cholesterol, increased SBP and alcohol consumption are the traditional risk factors that increase the risk CAD in T2DM patients. Among the newer risk factors, plasma MCP-1 might help to identify T2DM patients at risk of 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.

**Limitations of the present study**

The present study had some limitations. As this was a cross-sectional study, it was not possible to determine whether elevated or decreased levels of variables showing association with CAD actually preceded the development of CAD. Thus the clinical and laboratory variables found to have associations with complications in this study should only be interpreted as potential risk factors. Secondly it was a clinic based study hence there was a possibility of referral bias affecting the results. The exact chronological age of some patients in the present study could not be estimated due to lack of exact birth records among these patients. Although patients on anti-inflammatory drug therapy were not included in the present study, the probable effect of some anti-diabetic drugs on plasma MCP-1 and dM2-PK level cannot be completely ruled out.