CONCLUSION
The main finding in the present study was, patients with Type-II DM had a significantly higher rate of CAD than did non diabetic patients. As expected and previously shown, Type-II diabetes patients had more extensive CAD. DM appears as a strong predictor of CAD because of its multifactorial association.

The present study was first of its type in Central Indian population for CAD risk prediction and compression of different biochemical markers at one place, that provide a differential non invasive diagnostic approach towards CAD risk stratification.

The study also reported predictive power of conventional markers in comparisons to newer one, which had more value than newer one in developing country like India, reason could be if we could adjust them, we will be able to decrease epidemic of diabetes and CAD as well as socio economic burden.

Overall, identification of these individual risk factors had not only created a new challenge to the understanding of pathology of CAD and Type-II DM but also opened up approaches other than simply modifying the conventional risk factors in primary prevention of CAD.

The present study included many newer markers with conventional risk factors to their entire prominent role for the pathogenesis and occurrence of CAD in patients with or without Type II Diabetes mellitus.

The result revealed the following salient features.
1. Results of the present study revealed that Type II DM patients were at high risk for CAD, their variation CAD risk can be predicted moderately well by basic risk factors and a battery of novel markers.

2. Although patients with Type II DM having CAD were also found to have a good association of novel marker. Five novel risk markers greatly enhanced absolute CAD risk assessment. This investigation provided estimates of CAD risk for the primary prevention of this disease in our population.

3. It is observed that the increasing age with obesity which was a very common problem in the present population had a very positive impact on CAD occurrence with same prevalence of HTN, Smoking and FH of premature CAD.

4. Results suggested that NTproBNP is a strong predictor of CAD in type II diabetic patients and this association was independent of other CAD risk factors. The mechanism through which decrease in creatinine clearance increase the risk of CAD should be the focus of future research.

5. The activities of NO were significantly lower in diseased group, suggesting reduced production or more rapid catabolism of nitric oxide. Aging effect was a positive predictor of serum NO levels independently of other parameters including ADMA, which is the endogenous inhibitor of NOS enzyme.

6. The implication of present observations was that reduction in NO concentration increased the risk of CVD. Endothelial derived NO is the most potent endogenous vasodilator known, pharmacological inhibition
or a genetic deficiency of endothelial NO synthase impairs endothelial dependent vasodilatation and increases vascular resistance. In patients with diabetes vascular NO activity was reduced, leading to impaired endothelium – dependent vasodilatation and elevated platelet aggregation, finally CAD with ischemic syndrome.

7. ADMA is an endogenous and competitive inhibitor of NOS. Plasma level of this inhibitor was elevated in patients with atherosclerosis and in those with risk of atherosclerosis. By inhibiting NO production ADMA impair the blood flow, accelerates atherogenesis and interferes with angiogenesis. Thus reduced NO level seemed to play a central role in the development of ED amongst the multiple pathological mechanisms that had been postulated.

8. Type II Diabetes is associated with increase risk of CVD. Hyperglycemia is associated with ED in vivo and in vitro studies had shown it; therefore ED is an early feature in the development of vascular complications in people with diabetes. The present study provided evidence that elevated serum ADMA and reduced NO independently associated with CAD risk. ADMA has been associated with many traditional and novel risk factors in the setting of atherosclerosis, particularly hypertension, hyperlipidemia, and hyperhomocysteinemia. In addition, they are elevated in conditions of peripheral artery disease, stroke and end-stage renal failure.

9. ADMA is generated from the hydrolysis of ubiquitous proteins containing methylated arginine residues. The nuclear protein arginine N-methyltransferase (protein methylase I) has been shown to methylate internal arginine residues in a variety of proteins. The methyl groups may
be distributed symmetrically or asymmetrically to the guanidinium nitrogens of arginine, resulting in SDMA, N-monomethylarginine with ADMA being the predominant isomer. These methylated arginines are excreted in the urine. In addition, the metabolism of ADMA and N-monomethylarginine, but not SDMA, occurs via hydrolytic degradation to L-citruline and dimethylamine by DDAH. DDAH is an oxidant-sensitive enzyme, decline in DDAH activity was strongly associated with elevated ADMA levels in the plasma in vivo and in the conditioned medium in vitro. DDAH dysfunction hence seems plausible, especially in the setting of DM, in which hyperglycemia has been known to elevate oxidative stress.

Several pathways have been characterized to account for the increased production of free radicals in hyperglycaemia. For instance, elevated glucose may activate the polyol pathway, leading to the oxidation of sorbitol to fructose, coupled by the reduction of NAD to NADH. The increased ratio of NADH/NAD may in turn promote free-radical production by activating xanthine oxidase and inactivating intracellular and extra cellular SOD. It is possible that these processes contribute to reduced DDAH activity or glucose-induced oxidative stress, impaired DDAH activity leads to accumulation of ADMA instead of metabolizing into citrulline. This increased concentration of ADMA inhibits NOS resulting vasoconstriction and increased atherogeneity.

10. Elevated Hcy levels were seen in patients with CAD. Hyperhomocystenemia is associated with endothelial dysfunction. This effect of hyperhomocystenemia on endothelial cells is through ADMA. ADMA is metabolized to citrulline by the enzyme DDAH. The inhibition of DDAH by homocysteine leads to increase ADMA and decrease NO
production thus reduced DDAH activity produces gradual constriction of vessels.

11. Elevated LDL Cholesterol is another factor found to be associated with CAD risk along with increased ADMA activity. Human endothelial cells PRMT activity is up regulated by LDL-Cholesterol, due in part to the enhanced gene expression of PRMT. Thus we found ADMA intermeshed with both cholesterol and Hcy cardiovascular risk factors.

12. The last marker found to be associated significantly with CAD was MPO. MPO as described was not simply general marker of inflammation although it is a catalytic sink for NO and accelerator of ED. MPO and its reactive products, including hypochlorus acid, may have a number of additional adverse effects on endothelial function. For example hypochlorus acid may react with L-arginine, reducing the availability of substrate for NOS and forming chlorinated arginine species that inhibit the enzyme (NOS). MPO and hypochlorus acid also reduces the availability of NADPH, an essential cofactor for NOS. MPO has the capacity to nitrate proteins via production of reactive nitrogen species such as peroxynitrite and nitrogen dioxide, reactive nitrogen species have been shown to oxidatively modify LDL and render it atherogenic and uncouple NOS.

13. Reduced NO, an elevated ADMA, Hcy, MPO and LDL-c level were seen in CAD patients with or without diabetes mellitus which were appeared as independent predictors of adverse cardiac events in the present study and hence, are useful in the risk stratification of CAD patients. MPO had a higher sensitivity and NPV, Hcy had a higher
specificity and PPV, LDL-c had a good specificity, PPV and NPV with 85% of accuracy.

ADMA had second higher specificity, PPV with almost near overall diagnostic efficiency for prediction of an outcome of cardiac events. In conjunction with other markers decreased NO, increased ADMA, increased LDL-c, increased Hcy levels may provide valuable information in the future for identifying the high risk group in coronary artery disease in Type II DM. Overall accuracy testing of ADMA also supported the measurement of baseline ADMA, it is a good candidate test for inclusion among those used to evaluate atherosclerotic severity. Its diagnostic accuracy is nearly equivalent to conventional marker total cholesterol, LDL-c and newer marker Hcy, Lp(a) in patients with CAD.

14. Increased serum concentrations of ADMA were predictive of future CAD events in healthy, diabetic, population with moderate absolute risk. The association was independent of the conventional lipoprotein profile and other traditional risk factors for CAD. Thus the additional measurement of ADMA may improve prediction of atherosclerotic CAD complication. Further studies are warranted with controlled human trial to establish the clinical relevance of ADMA measurement in various stages of atherosclerotic process and to identify the specific pathophysiological mechanisms by which ADMA exerts its deleterious effects.

15. To conclude, the measurement of serum ADMA is a simple reliable fast method that can be performed on a single strip ELISA reader, now a day it is easily available in simple biochemical laboratory in developing country like India. A drawback of ELISA is that it needs proper washing steps whether manual or automatic, it needs constant invigilation. However, with due care and attention, this should be avoided. Further
studies need to be conducted to clarify the mechanism leading to the increased serum ADMA activity in cardiac diseases and the potential pathologic implications. However, a practical result of present study is in the demonstration of relatively simple ADMA and NO activity measurement could significantly improve the current efficiency of a laboratory’s evaluation of patients with suspected Type-II DM and CAD.

16. In conclusion, the present study firmly suggested for the first time in central Indian population that ADMA activity is adversely associated with a higher degree of endothelial alterations characteristic of the cardiovascular disease or CAD and NO activity is likely to be one of the vasoprotective, vasodilative mechanism involved in the vascular physiology.

17. The present study confirmed that there was an increased oxidative stress in diabetes compared to non-diabetes counterparts that emphasizes the importance of assessing these markers for early diagnosis and therapeutic interventions. As India is harboring the largest number of diabetics in the world and Indians being more susceptible to CAD than any other ethnic group makes it necessary to implement measures for the management of dual epidemics. It is also necessary to initiate primary preventive measures like reduction in serum lipids, avoiding smoking, increasing intake of arginine rich proteins, fruits and vegetables, physical activity, maintenance of healthy body weight and secondary preventive measures like control of hyperglycemia and HTN to mitigate the devasting consequences of diabetes leading to CAD.

18. Identification of these individual risk factors has not only created a new challenge to the understanding of pathology of CAD and Type-II
DM but has also opened up different approaches other than simply modifying the conventional risk factors in primary prevention of CAD. Therefore, the roles of newer risk factors have to be identified, data pertaining to this in Indian patient only. This study although having a sufficient sample size will go a long way in furthering the data on these new predictors.