Abstract

Type I Protein Arginine Methyltransferases catalyze the formation of Asymmetric Dimethyl Arginine (ADMA) residues by transferring methyl groups from S-Adenosyl-L-methionine to the guanidine groups of arginine residues in variety of eukaryotic proteins. In the previous studies it has been concluded that PRMT1 contributes the major type I protein arginine methyltransferases enzyme activity present in mammalian cells. ADMA is a naturally occurring inhibitor of NOS. It is clear that it is generated by many different cell types in the cardiovascular system and affects vascular and cardiac function. Correlation of ADMA with endothelial dysfunction and cardiovascular risk, together with the associations between cardiac risk factors and ADMA levels, suggest that ADMA is linked to cardiovascular disease, but strong causal relationships have yet to be established which qualifies it as a potential therapeutic target.
Introduction and Review of Literature:

Atherosclerosis has been a serious health epidemic in developed countries in the late 20th century, and its rising prevalence in developing nations suggests that it will become the chief cause of morbidity and mortality worldwide by early in the present century. Although the principal clinical complications of atherosclerosis, such as myocardial infarction and stroke, usually occur in middle-aged or older people, the atherogenic process actually begins in childhood and early adult life, with a preclinical phase lasting many decades. This pattern provides a window of opportunity for the high risk subjects, and the application of appropriate preventing strategies.

Diabetes and Atherosclerosis:

Both type 1 (insulin-dependent) and type 2 (non insulin-dependent) diabetic patients, have mostly been described under enhanced oxidative stress, and both conditions are known to be powerful and independent risk factors for coronary heart disease, stroke, and peripheral arterial disease.

The impressive correlation between coronary artery disease and alterations in glucose metabolism has raised the hypothesis that atherosclerosis and diabetes may share common antecedents. Large-vessel atherosclerosis can precede the development of diabetes, suggesting that rather than atherosclerosis being a complication of diabetes, both conditions may share genetic and environmental antecedents, a "common soil".
Published data suggest that abnormal endothelial function precedes other evidence of vascular disease and that the progression of metabolic syndrome to type 2 diabetes parallels the progression of endothelial dysfunction to atherosclerosis. Both type 1 and type II diabetes, like metabolic syndrome and other cardiovascular risk factors determine an abnormal endothelium response thought to precede the development of atherosclerosis.

Rationale for study of Various Markers:-

1) **Endothelial Dysfunction (ED):**

Dysfunction of the arterial endothelium is also an important early event with decreased local availability of nitric oxide and thus impaired vasodilator capacity. Inflammation and calcification of plaque may be slightly later events, but they still usually precede luminal narrowing and onset of the symptoms.

Here are different techniques to evaluate the endothelial functional capacity, that depend on the amount of NO produced and the vasodilatation effect. The percentage of vasodilatation with respect to the basal value represents the endothelial functional capacity. Taking into account that shear stress is one of the most important stimulant for the synthesis and release of NO, the non-invasive technique most often used is the transient flow-modulate "endothelium-dependent" post-ischemic vasodilatation, performed on conductance arteries such as the brachial, radial or femoral arteries. This vasodilatation is compared with the vasodilatation produced by drugs that are NO donors, such as nitroglycerine called "endothelium independent". The vasodilatation is quantified by measuring the arterial diameter with high resolution ultrasonography. Laser-Doppler techniques are now used that also
consider tissue perfusion. But we used angiography as a standard for measurement of vascular function and atherosclerosis. There is much proof about endothelial dysfunction that it is reasonable to believe that there is diagnostic and prognostic value in its evaluation for the late outcome. There is no doubt that endothelial dysfunction contributes to the initiation and progression of atherosclerotic disease and could be considered as an independent vascular risk factor.

Although prolonged randomized clinical trials are needed for unequivocal evidence, the data already obtained allows the methods of evaluation of endothelial dysfunction to be considered useful in clinical practice and have overcome the experimental step, being non-invasive increases its value making it useful for follow-up of the progression of the disease and the effects of different treatments.

2) Glycosylated/ Glycated Haemoglobin A1C (HbA1c), Fructosamine/Serum Glucose:

Hyperglycemia in diabetic patients causes glycosylation of proteins and phospholipids, thus increasing intracellular oxidative stress. Non-enzymatic reactive products, known as Maillard or browning reaction, glucose-derived Schiff base, and Ama-dori products, form chemically reversible early glycosylation products which subsequently rearrange to form more stable products, some of them on long-lived proteins (e.g. vessel wall collagen) and continue undergoing complex series of chemical rearrangements to form advanced glycosylation end products (AGEs) eg. Glycosylated Haemoglobin, Fructosamine. Once formed, AGEs are stable and virtually irreversible. AGEs generate ROS with consequent increased vessel oxidative damage. Measurement of these AGEs will help in early detection of disease.
3) **Highly Sensitive C-Reactive Protein (hs-CRP):**
Phagocytes have specialized receptors for AGEs, their activation leading to oxidation of lipoproteins, especially the phospholipid component in LDL, and stimulating an immune-inflammatory response and a thrombogenic response through Thromboxane A2 release and platelet aggregation induction. Diabetic patients have increased levels of inflammatory markers, including highly sensitive CRP, with pro-inflammatory and pro-atherogenic properties.

4) **Nitric Oxide (NO), Asymmetric Dimethyl Arginine (ADMA)**
Endothelial dysfunction and vascular inflammation contributes substantially to the accelerated atherogenesis associated with Type 2 diabetes. Endothelial derived Nitric Oxide (NO) is the primary compound responsible for vasodilatation in arteries and maintenance of normal vascular homeostasis. NO is synthesized from arginine by nitric oxide synthetase (NOS) in a complex reaction. Nitric Oxide cannot be measured directly but the inhibitor of its formation ADMA can be measured directly.

In patients with Type 2 diabetes, plasma ADMA is found to be increased, it may contribute to abnormal blood flow responses and to atherogenesis in Type 2 diabetes. Increased ADMA concentrations has been described in hypercholesterolemia, hypertension, arterial occlusive disease, patients prone to develop arteriosclerosis such as in Type 1 diabetes and Type 2 diabetes and in women with previous gestational diabetes (GDM) who are at risk of developing Type 2 diabetes. Insulin resistance was recently reported to be related to ADMA in non-diabetic normotensive subjects.

Human endothelial cells exposed to high glucose concentration show impairment of DDAH or dimethylarginine-dimethyl-amino-hydrolase and
accumulation of ADMA, that may contribute to endothelial vasodilator dysfunction in Diabetes Mellitus. There are findings which shows improved glycemic control in patients with Type 2 diabetes results in lowering plasma ADMA levels. Thus ADMA can be considered as an early biochemical marker for atherosclerosis as endothelial dysfunction and measured non invasively.

5) Homocysteine (Hey):
There is growing evidence that the well-known elevated Homocysteine with Cardiovascular disease is mediated by a mechanism involving ADMA or asymmetric dimethyl arginine, where homocysteine impairs the NO synthesis pathway.

Lowering average homocysteine concentrations by 3 micromole/l would reduce the risk of ischemic heart disease by 16%, deep vein thrombosis by 25% and stroke by 24%. Recent research indicates that the effect of homocysteine on cardiovascular disease is mediated by ADMA. By taking homocysteine one of the parameter in this study, it may state that Hey can be used as an early marker in relation to endothelial dysfunction as well as atherosclerosis with type 2 DM.

6) B-type natriuretic peptide:
NT-pro BNP belongs to a family of naturally occurring hormones known as B-type natriuretic peptides. They are secreted primarily by the ventricular myocardium in response to stress, including volume expansion and pressure overload. They provide reliable diagnostic and prognostic information but their release is not fully understood.
7) **Lipid Profile:**
In addition, the association of elevated LDL Cholesterol or low-density lipids with increased risk of heart disease may be as a co-variable in the oxidative activation of ADMA synthesis. Oxidized LDL particles stimulate the expression of enzyme that generates ADMA.

8) **Lipoprotein-a (Lp(a)):**
Lp(a) is a distinct class of lipoprotein that is structurally related to LDL, because both lipoprotein classes posses one molecule of apo B-100 per particle and have similar lipid compositions. However, unlike LDL, Lp(a) contains a carbohydrate-rich protein [apo(a)], Lp(a) contains one molecule of apo(a) and one molecule of apo B-100 per Lp(a) particle. Apo(a) is the unique protein component of Lp(a) and exhibits a significant sequence homology with plasminogen. Unlike plasminogen, however Lp(a) is not activated to form an active protease.
Several studies demonstrated that in people with CHD, changes in the serum concentrations of apo B-100 are similar to those for HDL and LDL respectively. Apo B-100 values were increased in people with diabetes and heart disease compare with those without disease. In most studies, apo B-100 were somewhat better discriminators of people with CHD than the cholesterol concentration of the corresponding lipoprotein.

9) **Myeloperoxidase (MPO):**
Myeloperoxidase (MPO) is a member of the heme peroxidase superfamily, first identified within human atherosclerotic plaque nearly a decade ago. MPO has emerged as an important potential participant in the atherosclerotic process, it may play a pathophysiological role in atherogenesis. Myeloperoxidase levels are higher in patients with
coronary artery disease and predict future cardiovascular events after risk factors.
MPO may also contribute to the atherosclerotic process by promoting endothelial dysfunction, by virtue of its capacity to catalytically consume nitric oxide as a substrate in vitro and in vivo, resulting in formation of nitric oxide–derived oxidants.
Moreover MPO predicts endothelial dysfunction more strongly than C-reactive protein suggesting that MPO (myeloperoxidase) is not simply a general marker of inflammation, but it has established relation between myeloperoxidase and cardiovascular disease which suggests its importance in atherosclerosis.
Recent human genetic studies support a potential role for MPO in CAD reporting MPO deficiency in subjects is cardioprotective and individuals possessing a functional polymorphism associated with approximately two-fold decrease in MPO expression have reduced cardiac risk.

10) Fibrinogen
Fibrinogen (FIB) is a soluble glycoprotein found in the plasma, with a molecular weight of 340 kDa. It comprises of three pairs of non identical polypeptide chains linked to each other by disulphide bonds. FIB has a biological half life of about 100 hour and is synthesized predominantly in the liver as a clotting factor; FIB is an essential component of the blood coagulation system, being the precursor of fibrin.
The relationship between hyperfibrinogenemia, atherosclerosis and thrombosis is complicated. As the process of thrombogenesis is very closely related to atheroma formation (atherogenesis), it follows that specific thrombogenic factors such as FIB may play key roles in the process of atherosclerotic lesion formation, with subsequent effects on CVD.
FIB and its metabolites appear to cause endothelial damage and dysfunction by a number of mechanisms. Many human atherosclerotic lesions, showing no evidence of fissure or ulceration, cavole contain a larger amount of fibrin, which may either be in the form of mural thrombus on the intact surface of the plaque. This phenomenon may be compounded by the decrease in arterial intimal fibrinolytic activity and plasminogen concentration observed in CAD.

11) AER
The US agency for health research and quality has recently reviewed the literature on increased albumin excretion and risk of renal disease and deteriorating mortality and found that the preponderance of evidence showed that increased albuminuria was associated with an increased risk of renal disease and cardiovascular mortality. Increased urinary albumin excretion reflects a generalised vascular dysfunction caused by structural alterations of the vascular wall, such as reduced content or sulphation of heparan sulphate within the extra cellular matrix. Such alterations may potentiate albuminuria and several of the processes involved in atherogenesis.

12) Ccr
Creatinine is the cyclic anhydride of creatine that is produced as the final product of decomposition of phosphocreatine. It is a waste product, it is filtered at the glomeruli and secreted by the tubules and excreted in urine. Lower creatinine clearances and higher serum creatinine concentration were found in patients with documented atherosclerosis.
Aim and objectives: -

The aim of present study is to find out the association of above mentioned markers with atherosclerosis in type II DM patients, to mention a non-invasive marker which is highly specific; highly sensitive that could be used as a prognostic and diagnostic tool for Atherosclerosis in CAD patients with or without Type-II DM.

Ethnic differences between populations have also been reported for the prevalence and risk factors of CAD on account of these differences. Studies on the risk factors for CAD in our population are of great importance. Here we are going to report the association of these markers and Atherosclerosis in large group of subjects from central Indian population.
Materials and Methods:-

The study was case-controlled in design. The patients have been selected as they were admitted in the hospital. Patients included in the present study were all admitted to the intensive coronary care unit (ICCU) or attending the out patient department of M. Y. Hospital attached to M.G.M. Medical College, Indore, Madhya Pradesh, and Chirayu Hospital and Heart speciality centre Bhopal, M.P., India, recruited from April 2007 to June 2009.

Three hundred individuals were included in the study and were divided into four groups. Group 1 (n=90) had no diabetes mellitus and no cardiovascular disease were grouped as NControl\textsuperscript{A}. Group 2 (n=70) had cardiovascular disease without history of diabetes mellitus, grouped as non diabetics with cardiovascular disease or NDwCAD\textsuperscript{B}. Group 3 (n=70) had diabetes mellitus only, grouped as diabetic control or Dcontrol\textsuperscript{C}. Group 4 (n=70) had both diabetes mellitus and cardiovascular disease, grouped as diabetics with cardiovascular disease or DwCAD\textsuperscript{D}.

All patients selected, were underwent medical examination by a physician. A careful medical history was taken to obtain information about other diseases (particularly hypertension, coronary heart disease, myocardial infarction, stroke, peripheral vascular disease, and endocrine disorders) and medication. Body weight and height were measured with subjects in light clothing without shoes. Three blood pressure recordings were obtained from the right arm while in the supine position after 30 min of rest at 5-min intervals, and their mean value was calculated. Blood samples were drawn into Vacutainer tubes.
Fully informed consent was obtained from patients and controls before the study was approved by the local ethical and research committee.

Group 1 and Group 3 were randomly selected from patients attending to medicine OPD of the institute, of them Group 1 subjects had negative history of CAD, Type-II DM or had normal resting ECG.

Type –II DM in the present study was defined as a fasting blood glucose level of $\geq 126.0$ mg/dl or post-prandial glucose level $\geq 200.0$ mg/dl or self reported physician diagnosis of diabetes, or pharmacological treatment for diabetes on the basis of mentioned criteria Group 3 subjects were selected for the study.

Subjects in Group 2 and Group 4 were recruited from inpatients and out patients departments of the institute. The diagnosis of CAD was made on the basis of clinical history (typical angina, history of myocardial infarction) and 12-lead standard ECG before subjecting them to coronary angiography. The presence of any stenosis $> 305\%$ according to coronary angiography by visual assessment of coronary artery was included in the study. In most of the cases patients had sustained the primary clinical event elsewhere and were then referred for further management.

Whole population was further categorized in two groups on the basis of CAD findings. Diseased Group (included Group 2 & Group 4) and Non Diseased Group (included Group 1 & Group 3).

**Risk factors for Cardio vascular disease**

In the present study, smoking was defined as regular smoking of cigarettes/beedies. Diabetes mellitus was diagnosed on the basis of
fasting blood glucose concentration of $\geq 126 \text{mg/dl}$ or a patient already on anti-diabetic medications. Systemic hypertension was considered to be present if the patient was taking anti-hypertensive treatment at the time of hospital admission or if blood pressure was recorded $\geq 140 \text{mm Hg}$ systolic and/or $\geq 90 \text{mm Hg}$ diastolic, at least twice on examination during admission. A positive family history of CAD was defined as first degree relative that had documented CAD $< 55$ years in males or $< 65$ years in females. For lipid analysis, samples were obtained after an overnight fast. Those patients whose body mass index is $\geq 25 \text{kg/m}^2$ were considered as obese. Patients who had serum concentration of total cholesterol (TC) $\geq 240 \text{mg/dl}$, or triglyceride (TG) $\geq 300 \text{mg/dl}$, or low-density lipoprotein cholesterol (LDL-C) $\geq 160 \text{mg/dl}$ or high-density lipoprotein cholesterol (HDL-C) $\leq 40.0 \text{mg/dl}$ or very-low-density lipoprotein cholesterol (VLDL-C) $\geq 40.0$ are considered as hyperlipidemics. NTproBNP levels $\geq 125.0 \text{pg/ml}$ was considered as higher or increased risk. Serum NO levels $\leq 30 \text{pmol/l}$ was considered as higher or increased risk. Serum ADMA levels $\geq 1 \text{pmol/l}$ were considered as higher or increased risk. Serum Hey $\geq 15 \text{pmol/l}$ were considered as higher or increased risk. Serum Lp(a) $\geq 30 \text{pmol/l}$ were considered as higher or increased risk. Hs-CRP levels $\geq 1 \text{mg/l}$ was considered as higher or increased risk. HbA1c $\geq 8\%$ was considered to uncontrolled diabetes.

**Collection of samples**

Venous blood was collected from all subjects between 9.00 am and 11.00 am soon after fasting from 10.00 pm in the previous day. Plain and EDTA vacutainer were used for blood collection. Serum was isolated by low-speed centrifugation. The samples were stored at $-20\degree\text{c}$ for $\leq 45$ days prior to analysis. This work was carried out in the M.Y.Hospital, Indore, M.P., India.
Biochemical analysis

1. Lipid profile done on fully automatic analyzer using a) Total cholesterol estimated by enzymatic, CHOD/PAP method Supplied by Roche Diagnostic Ltd. b) Triglyceride estimated by enzymatic, GPO/PAP method Supplied by Roche Diagnostic Ltd. c) High density lipoprotein estimated by enzymatic, CHOD/PAP method Supplied by Roche Diagnostic Ltd. d) Low density lipoprotein estimated by enzymatic, CHOD/PAP method Supplied by Roche Diagnostic Ltd. e) Very low density lipoprotein estimated by enzymatic, CHOD/PAP method Supplied by Roche Diagnostic Ltd.

2. Fasting blood sugar estimation done on fully automatic analyzer by using enzymatic assay kit.

3. NT-pro BNP was estimated on Elecsys 2010 fully automated immunoassays system by using pro BNP reagent kit, supplied by Roche Diagnostic Ltd.

4. NO (as nitrite) was estimated by kinetic cadmium reduction method kit supplied by Quantichrome.

5. ADMA was estimated on single plate ELISA reader using ELISA assay kit, Supplied by DLD Diagnostics.

6. MPO was estimated on single plate ELISA reader using ELISA assay kit, Supplied by DLD Diagnostics.

7. Homocysteine estimation by ELISA kits (Diazyme, San Diego, CA, USA).
8. hs-CRP estimation by ELISA (Biochek Inc USA).

9. Lp(a) by ELISA (US Biological, USA).


**Statistical Analysis**

All the data collected were statistically analyzed using the following tests where applicable: analysis of variance test (ANOVA) and chi-square test. To test the significance’s between the study and the control group, “X²” test and student t -test were used. The univariate analysis performed is identifying risk factors of CAD, Type-II DM, and severity of disease. The multivariate logistic regression analysis (stepwise) was performed taking significant risk factors at univariate analysis independent variables to identify the final set of risk factors. For both univariate and multivariate analysis, cut-off values were used for differentiating the presence or absence of the disease.

Statistical analysis analysis was carried out using SPSS 14.3 version. All data was reported as the mean ± SD or as a percentage. Demographic and clinical variables were compared by unpaired t test. Prevalence was compared by the χ² test. Correlation analysis was performed by means of spearman test. Statistical significance was defined as P < 0.05.

Present work was approved by institutional research and ethical committee.
Observations and Results:

Conventional risk factors analysis of whole cohort (n=300) is summarized in Table 1. Higher age and male sex was the commonest risk factor being present in subjects (60.66% and 82.66%). 43% patients were suffering from hypertension, 23% patients were with positive family history of CAD, while 22% patients were smokers.

After careful clinical examination and confirmed diagnosis, 70 patients presenting CAD (Group 2), 70 patients presenting Type-II DM only (Group 3) and 70 patients presenting Type-II DM with CAD (Group 4) were included in the study. The biochemical investigations were carried out in the blood samples of all the patients and age as well sex matched 90 healthy controls (Group 1). However, as was the study design, there was no diabetic in Group 1 and Group 2 patients whereas all the subjects in Group 3 and Group 4 had diabetes. Hence, the fasting and post-prandial blood glucose values (not shown) were higher in Group 3 and Group 4 patients compared to those in Group 1 and 2.

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1.1 Age and Sex

In the present study Group 2 patients belonged to the age group 50 to 70 years. Among Group 2 patients, 53 patients were male and 17 were females. The mean age in group 2 was 60.77 ± 9.93 years. In our study, Group 3 patients belonged to the age group of 43 to 63 years, with a mean age 52.91 ± 9.25. Among Group 3 patients, 61 patients were male and 09 were females. In the present study, Group 4 patients belonged to the age group of 53 to 71 years, the mean age was 62.24±9.22. Among Group 4 patients, 57 patients were male and 13 were females.

In the present study Group 1 subjects belonged to the age group of 43 to 57 years. Among Group 1 patients, 74 patients were male and 16 were females. The mean± SD of age in control was 50.22 ± 7.05 years. However, there was no significant difference of age between Group 2 compared to group 4 and Group 1 compared to Group 3 patients was observed. Even though there was a statistical difference (F: 133.66, p<0.001) among the patients and controls have been observed but overall population was age and sex matched as shown in Table 5.

1.2 Obesity, HTN

Nearly 7% subjects in Group1 were obese compared to Group 2 (24%, 22.5 ± 3.1), Group 3 (18%, 21.9 ± 2.4) and Group 4 (21%, 23.9 ± 1.7)
with a mean BMI of 23.4 ±1.4 for Group 1. The statistical difference was found to be p<0.001.

Higher SBP was more prevalent in patients (40%) with Group 4 compared to 11% in Group 3 patients, 31% in Group 2 patients and 5% in Group 1 controls which were significant statistically (p<0.001). Similarly DBP was more prevalent in patients (40%) with Group 4 compared to 11% in Group 3 patients, 28% in Group 2 patients and 4% in Group 1 controls which were also significant statistically (p<0.001).

1.3 Family history of CAD and Smokers

Positive family history of CAD was more prevalent significantly in patients (38% in Group 4 patients, 24% in Group 3 patients and 18% in Group 2 patients, p<0.001) compared with 16% of Group 1 subjects had positive family history. Nearly half of patients (43%) of Group 4 were smokers compared to 09% in Group 3 patients and 40% in Group 2 patients, there was no smoker found in Group 1 subjects. The statistical difference for smokers was p<0.001.

1.4 Serum Lipid Profile status

Lipid profile of patients and control subjects were presented in Table-7.

**Total Cholesterol:** - Group 4 patients had highest mean ± SD TC (270.22±42.53 mg/dl) levels. In comparison, the Group 1 subjects had significantly lower TC (146.4±27.19 mg/dl, p<0.001). Group 2 patients had significantly higher TC (247.48±34.25) levels compared to Group 1 and Group 3 patients (p<0.001). Also Group 4 patients had higher TC (270.22±42.53 mg/dl) levels compared to Group 3 TC (175.67±39.77 mg/dl, p<0.001). The increased TC (F: 109.05, p<0.001) levels were statistically highly significant.
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Triglyceride: - Group 4 patients had highest mean ± SD Triglyceride (260.21±49.00 mg/dl). In comparison, the Group 1 subjects had significantly lower Triglyceride (128.36±37.93mg/dl, p<0.001) levels. Group 2 and Group 3 patients had significantly higher Triglyceride (176.17±66.03 and 180.2±55.17) levels compared to Group 1 subjects. The increased Triglyceride (F: 116.77, p<0.001) levels. However, there were no significant differences of Triglyceride between Group 2 and Group 3 patients.

High Density Lipoprotein-cholesterol: - The mean ± SD value of HDL-c levels were 26.12±8.56 mg/dl in Group 4, 29.08±10.34 mg/dl in Group 2 compared to 42.57±7.78 mg/dl in Group 3 and 42.22±9.61 mg/dl in Group 1 patients, these reduced HDL-c levels was found to be statistically highly significant (F: 110.55, p<0.001) among all patients and controls.

Low Density Lipoprotein –cholesterol: - Group 4 patients had highest mean ± SD LDL-c (169.58±22.57mg/dl) levels. In comparison, the Group 1 subjects had significantly lower LDL-c (90.5±21.41 mg/dl, p<0.001). Group 2 patients had significantly higher LDL-c (153.47±28.25) levels compared to Group 1 and Group 3 patients (p<0.001). Also Group 4 patients had higher LDL-c (169.58±22.57mg/dl) levels compared to Group 3 LDL-c (109.92±28.32mg/dl, p<0.001). The increased LDL-c (F: 143.83, p<0.001) levels were statistically highly significant.

Very Low Density Lipoprotein –cholesterol: - The mean ± SD value of VLDL-c levels were 44.51±19.38 mg/dl in Group 2 and 44.28±12.14mg/dl in Group 4, highest in comparison to 34.41±8.80 mg/dl in Group 3 and 31.31±11.95 mg/dl in group 1. The increased
VLDL-c (F: 115.71, p<0.001) levels were statistically highly significant. However, there is no difference in between Group 2 and Group 4.

1.5 Comparison of hyperlipidemia status between different groups

The prevalence of hyperlipidemia in all the four groups is summarized in Table

Hypercholesterolemia (TC ≥ 250mg/dl) was seen in 78.25% patients in Group 2, 10% in Group 3 and 72.85% in Group 4. Of them Group 2 is having highest prevalence. More than 50% patients of Group 4 (65.71%) and Group 2 (52.85) patients had HDL-c level of ≤30mg/dl as compared to 10% in Group 1 and 5.71% in Group 3 (p<0.001)

It was also noted that about 80% of patients of Group 4 population had LDL-c level ≥ 150mg/dl while Group 3 had 11.40%. Group 2 was also had higher 68.57% of patients with VLDL-c level ≥ 150mg/dl (p<0.001). VLDL-c levels ≥39mg/dl were seen in about 57.14% of patients in Group 4 and 51.42% in Group 2 while it was significantly less common (32.85%) in Group 3 patients.

2 Serum Non –Lipid Markers

2.1 NT pro BNP

The NTproBNP levels were found to be highest in Group 4 patients (1600.09±808.66 pg/ml) compared to Group 2 and Group 3 Patients (707.76±357.94 pg/ml and 39.88±32.72 pg/ml) respectively as shown in Table 8. Whereas Group 1 patients had lowest NTproBNP (26.93±25.67 pg/ml) levels and which are statistically highly significant (F: 113.30, p<0.001). However, there was no significant difference of increased NTproBNP levels among Group 1 vs Group 3 patients.
2.2 NO

The levels of estimated NO were expressed in Table 8 for all the four groups. Group 1 had highest increased (54.36±8.09 μmol/L) levels as compared to Group 2 (31.014±5.005 μmol/L) levels and Group 3 (48±8.62 μmol/L) levels p<0.001. Whereas, Group had lowest NO (25.71±3.41 μmol/L) levels and which was statistically highly significant (F: 121.61, p<0.001). Group 3 was also having higher levels as compared to Group 2 and Group 4, which was found to be statistically highly significant (p<0.001).

2.3 ADMA

ADMA levels estimated in CAD patients, Type-II DM patients, patients with CAD Type-II DM and normal healthy subjects were presented in Table 8. Group 4 patients had elevated ADMA (67.52±9.83 μmol/L) levels, similarly Group 2 patients also had elevated ADMA (66.54±7.062 μmol/L) levels as compared to Group 3 patients (46.81±13.20 μmol/L) levels. Levels of ADMA (34.08±9.78 μmol/L) in Group 1 patients was lowest in all four groups which was statistically highly significant (F: 137.71, p<0.001). There is slight elevation in Group 3 patients ((46.81±13.20 μmol/L) levels as compared to Group 1 subject’s levels.

2.4 MPO

MPO levels were estimated in all four Groups of subjects and were presented in Table 8. The mean ± SD of MPO levels in serum of Group 2 patients was found to be 691.3±174.63 μmol/L and Group 3 patients had 465.81±195.78 μmol/L. When compared to these two Group patients with Group 4 had highest MPO (862.12±206.61 μmol/L) levels and Group 1 subjects had lowest MPO (275.07±64.49 μmol/L) levels respectively and were statistically highly significant (F: 150.58, p<0.001).
2.5 Homocystein

The Hcy levels were found to be highest in Group 4 patients (16.14±1.50 μmol/L) compared to Group 3 and Group 2 patients (7.008±2.045 μmol/L and 15.57±1.54 μmol/L) levels. Group 1 patients had lowest Homocystein (6.56±2.16 μmol/L) levels with statistically significant (F: 142.23, p<0.001) value. However there is no difference between Group 1 and Group 3 patient’s levels as well as Group 2 and Group 4 patient’s levels.

2.6 Lp(a)

Lp(a) activity was estimated in patients with CAD, patients with Type-II DM and CAD with Type-II DM patients along with normal healthy controls and listed in Table 8. Among patients Group 4 had highest Lp(a) (53.80±29.25 mg/dl) activities compared to Group 3 patients (24.28±5.35 mg/dl) and Group 2 patients (38.17±25.50 mg/dl). But in case of Group 1 subjects, they were had lowest Lp(a) (11.69±6.54 mg/dl) activity and found to be statistically highly significant (F: 134.80, p<0.001). Lp(a) levels of Group 2 patients (38.17±25.50 mg/dl) were significantly higher as compared to Group 1 and group 3 (p<0.001) patients.

2.7 hs-CRP

hs-CRP levels estimated for whole population studied were present in Table 2. Group 4 patients had elevated hs-CRP (1.10±1.10 mg/L) levels compared to group 3 patients (0.610±0.266 mg/L) and Group 2 patients (0.90±1.24 mg/L). Whereas Group 1 subjects had lowest hs-CRP (0.262±0.150 mg/L) levels and were found to be statistically highly significant (F: 69.24, p<.001). hs-CRP levels of Group 2 patients (0.90±1.24 mg/L) were significantly higher as compared to Group 1 and Group 3 (p<0.001) patients.
2.8 Fibrinogen

Fibrinogen levels were estimated in all four Groups of subjects and were presented in Table 8. The mean ± SD of Fibrinogen levels in serum of Group 2 patients was found to be 325.78±84.85 mg/dl and Group 3 patients had 304.23±92.07 mg/dl. When compared to these two Group patients with Group 4 had highest Fibrinogen (411.83±89.90 mg/dl) levels and Group 1 subjects had lowest Fibrinogen (177.66±59.19 mg/dl) levels respectively and were statistically highly significant (F: 143.69, p<0.001). However there is no difference between Group 2 and Group 3 patient’s levels.

2.9 Glycated haemoglobin A1c

The HbA1c levels were found to be highest in Group 4 patients (9.29±1.16%) compared to Group 3 and Group 1 patients (7.01±0.57 % and 4.94±0.63%) levels. Group 2 patients had lowest HbA1c (4.85±0.57%) levels with statistically significant p<0.001 value. However there is no difference between Group 1 and Group 2 patient’s levels.

2.10 Serum Fructosamine and Globin Bound Fructosamine

The Serum Fructosamine levels were found to be highest in Group 3 patients (3.65±5.27 mmol/L) compared to Group 2 and Group 1 patients (1.61±0.22 mmol/L and 1.94±2.74 mmol/L) levels. Group 4 patients had second highest Serum Fructosamine levels (2.49±0.45 mmol/L) levels with statistically significant (F: 41.84, p<0.001) value. However there is no difference between Group 1 and Group 2 patient’s levels.

Group 3 had lowest levels of Globin Bound Fructosamine compared to Group 4, there is similar values for Group 1 and Group 2 of Globin
Bound Fructosamine with a F: 83.23 value which is significant statistically p<0.001.

2.11 Creatinine clearance rate
Ccr levels estimated in twenty four hour urine samples of CAD patients, Type-II DM patients, patients with CAD Type-II DM and normal healthy subjects were presented in Table 8. Group 4 patients had lowest Ccr (57.61±11.63 ml/minx1.73m) levels, similarly Group 2 patients also had decreased Ccr (60.34±6.85 ml/minx1.73m) levels as compared to Group 1 patients (79.21±4.31 ml/minx1.73m) levels. Levels of Ccr (85.39±27.09 ml/minx1.73m) in Group 3 patients and was found to be highest as compared to other groups studied, which was statistically highly significant (F: 107.77,p<0.001).

2.12 Albumin Excretion Rate
AER was estimated in twenty four hour urine samples of all four Groups of subjects and were presented in Table 8. The AER were found to be highest in Group 4 patients (49.66±22.34 mg/day) compared to Group 3 and Group 2 patients (33.06±10.22 mg/day and 19.02±9.58 mg/day) albumin excretion rate. Group 1 patients had lowest AER (15.48±8.86 mg/day) levels with statistically significant p<0.001(F: 166.32) value.

2.13 ESR
ESR was estimated in whole blood samples of CAD patients, Type-II DM patients, patients with CAD Type-II DM and normal healthy subjects were presented in Table 8. The Erythrocyte sedimentation rate was found to be highest in Group 4 patients (31.89±7.56 ml/h) compared to Group 3 patients (18.55±2.18 ml/h). Group 1 patients had lowest ESR (17.95±1.45 ml/h) levels with statistically significant (F: 143.97, p<0.001) value.
However there is no difference in between Group 1 and Group 3, similarly Group 2 and Group 4.

3 Univariate analysis of the subjects

The univariate analysis was performed to identify the risk factors for CAD. The univariate logistic regression analysis for risk factors versus CAD (as dependent variable) was done to assess the relative risk of development of CAD with each risk factor in the entire subject group which includes 140 patients and 160 controls. There was an association of more than one risk factor in most of the subjects. Thus, regression analysis was used to estimate the risk association of each risk factor. It was estimated using the cut-off values (as a marker) for each biochemical parameters.

Age >50 years as risk factor contributed significantly ($X^2$: 17.67, p<0.001) to the pathogenesis of CAD in patients. Gender as risk factor contributed not significantly ($X^2$: 0.421, p<0.61) to the pathogenesis of CAD in patients. However, positive family history was contributed significantly ($X^2$: 17.15, p<0.001). On analyzing the role of conventional risk factors in affecting the pathogenesis of CAD in patients population we found that, among all subjects, obesity ($X^2$: 10.28, p<0.05) was significantly associated with the occurrence of CAD. In addition, SBP ($X^2$: 39.09, p<0.001), DBP ($X^2$: 42.55, p<0.001) and smoking ($X^2$: 41.71, p<0.001) were statistically highly significant and they are associated with CAD pathogenesis. Refer Table 9 and 10.
Biochemical Parameters

3.1 Total Cholesterol

The cut-off level of TC is 250 mg/dl. Below this value suggests that the absence of disease and above this value explains the presence of the disease. In our study, Group 1 subjects (100%) had <250 mg/dl TC values as compared to all groups of patients. 78% of the Group 2 patients and 10% of the Group 3 patients were had >250 mg/dl. However, majority of the patients of the Group 4 (73%) had >250 mg/dl and by comparing all subjects. TC becomes an independent risk factor and their increased levels was significantly (p<0.001) associated with the pathogenesis of CAD in patients as presented in Table 11.

3.2 Triglycerides

The cut-off level of TC is 200 mg/dl. Below this value suggests that the absence of disease and above this value explains the presence of the disease. In our study, Group 1 subjects (100%) had <200 mg/dl TC values as compared to all groups of patients. 27% of the Group 2 patients and 27% of the Group 3 patients were had >200 mg/dl. However, majority of the patients of the Group 4 (90%) had >200 mg/dl and by comparing all subjects. These results shows that Triglyceride becomes an independent risk factor and their increased levels was significantly (p<0.001) associated with the pathogenesis of CAD in patients as presented in Table 11.

3.3 HDL-cholesterol

The cut-off level of HDL-c is 30 mg/dl. Below this value suggests that the presence of disease and above this value explains the absence of the disease. In our study, Group 1 subjects (10%) had <30 mg/dl HDL-c values as compared to all groups of patients. 53% of the Group 2 patients
and 6% of the Group 3 patients were had <30 mg/dl. However, majority of the patients of the Group 4 (66%) had <30 mg/dl and by comparing all subjects. These lines exhibit that decreased HDL-c becomes an independent risk factor and their increased levels was significantly (X²: 51.56 p<0.001) associated with the pathogenesis of CAD in patients as presented in Table 11.

3.4 LDL-cholesterol
The cut-off level of LDL-c is 150 mg/dl. Those subjects had less than 150 mg/dl show the absence of disease. On the other hand, patients who had more than 150 mg/dl LDL-c indicate the presence of the disease. Almost 75% of the Group 1 subjects had <150 mg/dl LDL-c levels, which shows absence of the disease. Amongst patients, majority of Group 4 (80%) had >150 mg/dl of LDL-c. More than half of Group 2 patients (68%) had >150 mg/dl of LDL-c. In group 3 only 12% patients had >150 mg/dl of LDL-c. By comparing all subjects, increased level of LDL-c becomes independent risk factor and significantly (X²: 69.79, p<0.001) associated with the occurrence of the CAD.

3.5 VLDL-cholesterol
The cut-off level of VLDL-c is 40 mg/dl. Those subjects had less than 40 mg/dl show the absence of disease. On the other hand, patients who had more than 40 mg/dl VLDL-c indicate the presence of the disease. All of the Group 1 subjects had <40 mg/dl VLDL-c levels, which shows absence of the disease. Amongst patients, majority of Group 4 (57%) had >40 mg/dl of VLDL-c. More than half of Group 2 patients (52%) had >40 mg/dl of VLDL-c. In group 3 only 33% patients had >40 mg/dl of VLDL-c. By comparing all subjects, increased level of VLDL-c becomes
independent risk factor and significantly ($X^2: 25.52, p<0.001$) associated with the occurrence of the CAD.

3.6 **NT pro BNP**

The cut-off value is 125pg/ml, less than this value indicates the absence of disease but more than this value indicates the presence of disease. None of the Group 1 and Group 3 patients had >125 pg/ml of NTproBNP, it represents disease free state of patients. All patients of Group 2 and Group 4 had >125 pg/ml of NTproBNP levels, it demonstrated that NTproBNP is an independent risk factor ($p<0.001$) associated with CAD.

3.7 **Nitric oxide**

In this study the cut-off levels of NO was 30 μmol/l. Below this cut-off value was considered as presence of disease and above this cut-off value considered as absence of disease. Majority of Group 1 subjects (93%) had >30 μmol/l of NO levels suggesting absence of disease. Amongst patients Group 2 had (78%), Group 3 had (45%) and Group 4 had (91%) patients with <30 μmol/l of NO levels which indicates presence of disease. These data supports that, NO is also one of the independent risk factor and significantly ($X^2: 40.05, p<0.001$) associated with the pathogenesis of the CAD.

3.8 **Assymetric dimethyl arginine**

In the present study the cut-off levels of ADMA was 60 μmol/l. The subjects had less than this cut-off value was considered as absence of disease and the patients had more than this cut-off value considered as presence of disease. No one subject of Group 1 had >60 μmol/l of ADMA levels suggesting absence of disease. Amongst patients Group 2 had
(74%), Group 3 had (15%) and Group 4 had (67%) patients with >60 µmol/L of ADMA levels which indicates presence of disease. These results reflects that, ADMA is one of the strongest independent risk factor and significantly ($X^2$: 36.06, $p<0.001$) associated with the pathogenesis of the CAD.

3.9 **Mycoperoxidase**

The cut-off value is 300 µmol/l, less than this value indicates the absence of disease but more than this value indicates the presence of disease. 63% of the Group 1 subjects had >300 µmol/l of MPO, Group 2 (90%) patients had >300 µmol/l of MPO, Group 3 (75%) of elevated MPO and 92% of Group 4 had >300 µmol/l of MPO it represents presence of disease in patients. These lines represents MPO as a risk factor ($X^2$: 17.80, $p<0.001$) which is independently associated with CAD.

3.10 **Homocysteine**

In this study the cut-off levels of Hcy was 15 µmol/l. Below this cut-off value was considered as absence of disease and above this cut-off value considered as presence of disease. Majority of Group 1 subjects (96%) had <15 µmol/l of Hcy levels suggesting absence of disease. Amongst patients Group 2 had (66%), Group 3 had (2%) and Group 4 had (73%) patients with >15 µmol/l of Homocystein levels which indicates presence of disease. These data supports that, Homocystein as one of the independent risk factor and significantly ($p<0.005$) associated with the pathogenesis of the CAD. However there was no difference in Hcy levels between Group 1 and Group 3.
3.11 Lipoportien(a)

The cut-off level of Lp(a) is 30 mg/dl. Those subjects had less than 30 mg/dl show the absence of disease. On the other hand, patients who had more than 30 mg/dl Lp(a) indicate the presence of the disease. Majority of the Group 1 (98%) subjects had <30 mg/dl Lp(a) levels, which shows absence of the disease. Amongst patients, majority of Group 4 (87%) had 340 mg/dl of Lp(a). More than half of Group 2 patients (60%) had >30 mg/dl of VLDL-c. In group 3 only 10% patients had >30 mg/dl of Lp(a). By comparing all subjects, increased level of VLDL-c becomes independent risk factor and significantly ($X^2$: 31.04, p<0.001) associated with the occurrence of the CAD.

3.12 Hs-CRP

The cut-off value is 1 mg/l, less than this value indicates the absence of disease but more than this value indicates the presence of disease. 100% of the Group 1 subjects had <1mg/l of hs-CRP, Group 2 (12%) patients had >1 mg/l of hs-CRP, Group 3 (10%) of elevated hs-CRP and 41% of Group 4 had >1 mg/l of hs-CRP it represents presence of disease in patients. These lines represents hs-CRP as a risk factor ($X^2$:20.86, p<0.001) which is independently associated with CAD.

3.13 HbA1c

The cut-off level of HbA1c is 8%. Those subjects had less than 8% show the absence of disease. On the other hand, patients who had more than 8% HbA1c indicate the presence of the disease. No one of the Group 1 subjects had >8% of HbA1c levels, which shows absence of the disease. Amongst patients, majority of Group 4 (91%) had >8% of HbA1c. Only one of the Group 3 patients had more than 8% of HbA1c Group 2 patients were free from elevated levels of HbA1c. By comparing all subjects,
increased level of HbA1c becomes a risk factor and significantly ($\chi^2$: 37.04, $p<0.001$) associated with the occurrence of the CAD.

### 3.14 Serum Fructosamine

The median value was taken as cut-off level of Serum Fructosamine is 0.018 ΔA/min. Those subjects had less than 0.018 ΔA/min show the absence of disease. On the other hand, patients who had more than 0.018 ΔA/min of Serum Fructosamine indicate the presence of the disease. Only 19% of Group 1 subjects had 0.018 ΔA/min of Serum Fructosamine levels. Amongst patients, majority of Group 4 (91%) had 0.018 ΔA/min of Serum Fructosamine. Group 3 patients had (61%) >0.018 ΔA/min as compared to Group 2 patients (19%) elevated levels of serum Fructosamine. There was no significant difference found.

### 3.15 Globin Bound Fructosamine

The median value was taken as cut-off level of Globin Bound Fructosamine is 2.8 mmol/l. Those subjects had less than 2.8 mmol/l show the absence of disease. On the other hand, patients who had more than 2.8 mmol/l of Globin Bound Fructosamine indicate the presence of the disease. Only 23% of Group 1 subjects had >2.8 mmol/l of Globin Bound Fructosamine levels. Amongst patients, majority of Group 4 (100%) had <2.8 mmol/l of Globin Bound Fructosamine. Group 3 patients had (70%) >2.8 mmol/L as compared to Group 2 patients (20%) elevated levels of Globin Bound Fructosamine. There was no significant difference found.

### 3.16 Ccr

The cut-off value is 146 ml/minx1.73m, less than this value indicates the presence of disease but more than this value indicates the absence of
disease. None of the Group 1 and Group 2 patients had <146 ml/minx1.73m of Ccr, it represents disease free state of patients. (8%) of Group 2 and (42%) Group 4 had > 146 ml/minx1.73m of Ccr levels, it demonstrated that Ccr is an independent risk factor ($X^2:25.93, p<0.001$) associated with CAD.

3.17 AER

In this study the cut-off levels of AER was 30 mg/dl. Below this cut-off value was considered as absence of disease and above this cut-off value considered as presence of disease. Majority of Group 1 subjects (80%) had <30 mg/dl of AER levels suggesting absence of disease. Amongst patients Group 2 had (18%), Group 3 had (47%) and Group 4 had (70%) patients with >30 mg/dl of AER levels which indicates presence of disease. These data supports that, AER as one of the risk factor and significantly ($X^2:11.19, p<0.01$) associated with the pathogenesis of the CAD.

3.18 ESR

The cut-off level of HbA1c is 20 ml/h. Those subjects had less than 20 ml/h show the absence of disease. On the other hand, patients who had more than 20 ml/h of ESR indicate the presence of the disease. Majority of Group 1 subjects (93%) had <20 ml/h ESR levels suggesting absence of disease. Amongst patients Group 2 had (89%), Group 3 had (64%) and Group 4 had (90%) patients with >20 ml/h of ESR levels which indicates presence of disease. These data supports that, ESR as one of the risk factor and significantly ($X^2:31.79, p<0.01$) associated with the pathogenesis of the CAD.
3.19 Fibrinogen

In this study the cut-off levels of Fibrinogen was 300 mg/dl. Below this cut-off value was considered as absence of disease and above this cut-off value considered as presence of disease. Majority of Group 1 subjects (93%) had <300 mg/dl of Fibrinogen levels suggesting absence of disease. Amongst patients Group 2 had (69%), Group 3 had (59%) and Group 4 had (85%) patients with >300 mg/dl of Fibrinogen levels which indicates presence of disease. These data supports that, Fibrinogen is also one of the independent risk factor and significantly ($X^2$: 64.76, $p<0.001$) associated with the pathogenesis of the CAD.

4 Multivariate analysis of the subjects

The multivariate logistic regression analysis was performed by taking risk factors at univariate analysis as independent variables to identify the final set of risk factors. This multivariate regression analysis for risk factors versus CAD revealed that age as risk factor and it will contributed significantly ($t$:9.64, $p<0.003$) associated with the occurrence of CAD. However, sex is also contributed to the CAD but it was not statistically significant ($t$: 0.49, $p=0.622$) as shown in Table -21.

Conventional risk factor such as positive family history of CAD and smoking was found to be not significant statistically ($t$: 1.862, $P<0.60$ and $t$: 0.700, $p<0.48$) contributed with the occurrence of CAD on multivariate analysis. However, HTN is one of the main risk factor for CAD and Type-II DM. In this study, univariate analysis shows SBP and DBP was significantly associated with disease mentioned. But in multivariate analysis, DBP was not statistically significant ($t$: 0.533, $p=0.595$) with the occurrence of CAD, SBP is associated with CAD but less significantly ($t$: 1.99, $p<0.047$). Obesity as a risk factor which was significantly ($t$: 5.234, $p<0.001$) associated with the CAD.
On multivariate analysis of lipid parameters we found, increased TC levels, elevated TG levels and HDL-c are weak predictors of CAD (t: 2.29, p<0.023, t: 1.75, p<0.080 and t: 2.30, p<0.022) but significant at the p<0.05 levels. However, reduced HDL-c was found to be associated with the atherogenesis. Instead LDL-c was highly associated with CAD occurrence (t: 3.64) and highly significant (p<0.001) statistically.

Turning towards non lipid biochemical parameters, increased NT pro BNP concentration was to be highly significant (t: 7.26, p<0.001) contributed with progression of CAD. Elevated NO levels was strong predictor and significantly (t: 9.95, p<0.001) associated with the occurrence of CAD. Increased ADMA level was found to be another strong predictor and associated significantly (t: 7.92, p<0.001) with CAD. Elevated MPO level was become strapping risk factor and statistically significant (t: 2.99, p<0.004) with the pathogenesis of CAD.

Multivariate analysis for Homocystien reveals that, increased Hcy concentration was significantly (t: 10.51, p<0.001) play an important role in progression of CAD in patients. While there was no significant contribution of Lp (a) statistically (t: 0.868, p=0.386).

Multivariate Analysis of Glycated protein as a risk factor shows, increased HbA1c levels associated with CAD occurrence with (t: 2.23, p<0.026) value. However there was no significant role of Serum Fructosamine and Globin bound fructosamine (t: 0.438, p=0.662 and t: 0.456, p=0.649) found. Fibrinogen plays no significant role (t: 0.383, p=0.702) in pathogenesis of CAD. While some previous study shows its role in CAD occurrence.

Of inflammatory risk markers Creatinine clearance rate is highly (t: 5.10, p<0.001) associated with CAD prevalence. Whereas Albumin excretion rate and Erythrocyte sedimentation rate had no significant (t: 0.371, p=0.711 and t: 1.181, p=0.239) role in CAD incidence.
After performing both univariate and multivariate logistic regression analysis for all risk factors against disease revealed that several risk factors and biochemical markers are contributed significantly to the pathogenesis of CAD and Type-II DM. Amongst, elevated TC, TG, LDL-c and reduced HDL-c were well known markers for the occurrence of disease. Therefore, an attempt was made in the present study to look novel biochemical markers like NO, ADMA, MPO, Hcy, Lp(a), hs-CRP and Glycated protein as good and better predictors of CAD. As there is no precise scientific data available towards role of reduced NO, elevated ADMA and their role in pathogenesis of CAD, an attempt has been made to look in to these aspects carefully.

5 Diagnostic Validity Testing
Apart form univariate and multivariate regression analysis, diagnostic validity for all parameter were done to assess a good and better novel predictor for CAD, referring Table 26, 27, 28.

Sensitivity (presence of the disease if ≤30µmol/L) of NO was found to be 20% for Group 2 patients, 7.1% for Group 3 and 85% for Group 4 patients. Specificity (absence of the disease if ≤30 µmol/L) of NO was found to be 94% for Group 2, Group 3 and Group 4 patients. PPV (positive predictive value) of NO was 78% Group 2 patients, 46% for Group 3 and 91% for Group 4 patients. NPV (negative predictive value) of NO was found to be 60% for Group 2 patients, 56% for Group 3 and 89% for Group 4 patients. So that, overall Accuracy (OA) or diagnostic efficiency (DE) of NO was found to be 61% for Group 2 patients, 56% for Group 3 and 90% for Group 4 patients.

Sensitivity (presence of the disease if ≥30µmol/L) of ADMA was found to be 74% for Group 2 patients, 14% for Group 3 and 67% for Group 4....
patients. Specificity (absence of the disease if $\geq 30 \, \mu\text{mol/L}$) of ADMA was found to be 100% for Group 2, Group 3 and Group 4 patients. PPV (positive predictive value) of ADMA was 100% for all groups (Group 2, Group 3 and Group 4 patients). NPV (negative predictive value) of ADMA was found to be 83% for Group 2 patients, 40% for Group 3 and 80% for Group 4 patients. So that, overall Accuracy (OA) or diagnostic efficiency (DE) of ADMA was found to be 61% for Group 2 patients, 56% for Group 3 and 90% for Group 4 patients.

Sensitivity (presence of the disease if $\leq 300 \, \mu\text{mol/l}$) of MPO was found to be 90% for Group 2 patients, 79% for Group 3 and 91% for Group 4 patients. Specificity (absence of the disease if $\leq 300 \, \mu\text{mol/l}$) of MPO was found to be 51% for Group 2, Group 3 and Group 4 patients. PPV (positive predictive value) of MPO was 59% Group 2 patients, 56% for Group 3 and 60% for Group 4 patients. NPV (negative predictive value) of MPO was found to be 87% for Group 2 patients, 75% for Group 3 and 89% for Group 4 patients. So that, overall Accuracy (OA) or diagnostic efficiency (DE) of MPO was found to be 68% for Group 2 patients, 63% for Group 3 and 69% for Group 4 patients.

Sensitivity (presence of the disease if $\geq 15 \, \mu\text{mol/l}$) of Hcy was found to be 66% for Group 2 patients, 02% for Group 3 and 73% for Group 4 patients. Specificity (absence of the disease if $\geq 15 \, \mu\text{mol/l}$) of Hcy was found to be 97% for Group 2, Group 3 and Group 4 patients. PPV (positive predictive value) of Hcy was 94% Group 2 patients, 25% for Group 3 and 95% for Group 4 patients. NPV (negative predictive value) of Hcy was found to be 78% for Group 2 patients, 56% for Group 3 and 82% for Group 4 patients. So that, overall Accuracy (OA) or diagnostic efficiency (DE) of Hcy was found to be 78% for Group 2 patients, 56% for Group 3 and 82% for Group 4 patients.
efficiency (DE) of Hey was found to be 83% for Group 2 patients, 55% for Group 3 and 86% for Group 4 patients.

Sensitivity (presence of the disease if ≥30 mg/dl) of Lp(a) was found to be 60% for Group 2 patients, 10% for Group 3 and 86% for Group 4 patients. Specificity (absence of the disease if ≥30 mg/dl) of Lp(a) was found to be 98% for Group 2, Group 3 and Group 4 patients. PPV (positive predictive value) of Lp(a) was 95% Group 2 patients, 78% for Group 3 and 97% for Group 4 patients. NPV (negative predictive value) of Lp(a) was found to be 76% for Group 2 patients, 58% for Group 3 and 90% for Group 4 patients. So that, overall Accuracy (OA) or diagnostic efficiency (DE) of Lp(a) was found to be 81% for Group 2 patients, 59% for Group 3 and 92% for Group 4 patients.

Sensitivity (presence of the disease if ≥1.00mg/L) of hs-CRP was found to be 11% for Group 2 patients, 10% for Group 3 and 41% for Group 4 patients. Specificity (absence of the disease if ≥1.00mg/L) and PPV (positive predictive value) of hs-CRP was found to be 100% for Group 2, Group 3 and Group 4 patients. NPV (negative predictive value) of hs-CRP was found to be 59% for Group 2 patients, 59% for Group 3 and 69% for Group 4 patients. So that, overall Accuracy (OA) or diagnostic efficiency (DE) of hs-CRP was found to be 62% for Group 2 patients, 65% for Group 3 and 75% for Group 4 patients.

Sensitivity (presence of the disease if ≥146 ml/minx1.73m) of Ccr was found to be 11% for 8.5% for Group 3 and 41% for Group 4 patients. Specificity (absence of the disease if ≥146 ml/minx1.73m) and PPV (positive predictive value) of Ccr was found to be 100% for Group 3 and Group 4 patients. NPV (negative predictive value) of Ccr was 59% for Group
3 and 69% for Group 4 patients. So that, overall Accuracy (OA) or diagnostic efficiency (DE) of Ccr was found to be 09% for Group 3 patients, 63% for Group 4 patients.

Sensitivity (presence of the disease if \( \geq 30 \text{ mg/day} \)) of AER was found to be 19% for Group 2 patients, 47% for Group 3 and 70% for Group 4 patients. Specificity (absence of the disease if \( \geq 30 \text{ mg/day} \)) of AER was found to be 94% for Group 2, Group 3 and Group 4 patients. PPV (positive predictive value) of AER was 72% Group 2 patients, 87% for Group 3 and 91% for Group 4 patients. NPV (negative predictive value) of AER was found to be 60% for Group 2 patients, 70% for Group 3 and 80% for Group 4 patients. So that, overall Accuracy (OA) or diagnostic efficiency (DE) of AER was found to be 61% for Group 2 patients, 74% for Group 3 and 84% for Group 4 patients.

Sensitivity (presence of the disease if \( \geq 300 \text{ mg/dl} \)) of Fibrinogen was found to be 69% for Group 2 patients, 59% for Group 3 and 89% for Group 4 patients. Specificity (absence of the disease if \( \geq 300 \text{ mg/dl} \)) of Fibrinogen was found to be 92% for Group 2, Group 3 and Group 4 patients. PPV (positive predictive value) of Fibrinogen was 87% Group 2 patients, 85% for Group 3 and 89% for Group 4 patients. NPV (negative predictive value) of Fibrinogen was found to be 79% for Group 2 patients, 74% for Group 3 and 89% for Group 4 patients. So that, overall Accuracy (OA) or diagnostic efficiency (DE) of Fibrinogen was found to be 82% for Group 2 patients, 77% for Group 3 and 89% for Group 4 patients.

Sensitivity (presence of the disease if \( \geq 20 \text{ ml/h} \)) of ESR was found to be 70% for Group 2 patients, 16% for Group 3 and 87% for Group 4 patients.
patients. Specificity (absence of the disease if ≥20 ml/h) of ESR was found to be 93% for Group 2, Group 3 and Group 4 patients. PPV (positive predictive value) of ESR was 89% Group 2 patients, 64% for Group 3 and 91% for Group 4 patients. NPV (negative predictive value) of ESR was found to be 80% for Group 2 patients, 59% for Group 3 and 90% for Group 4 patients. So that, overall Accuracy (OA) or diagnostic efficiency (DE) of ESR was found to be 83% for Group 2 patients, 59% for Group 3 and 95% for Group 4 patients.

On analyzing all novel markers levels with respect to occurrence of outcome measures, levels of Lp(a), NO, ADMA, MPO, Hcy, NTproBNP, Ccr and ESR were found to be more considerable between Group 2, Group 3 and Group 4. When similar diagnostic validity testing performed between Diseased group and Non diseased group similar results were found and shown in Table 28. Overall a comparison was made by considering sensitivity, specificity and accuracy of above mentioned markers for CAD and it was found that Lp(a) was emerged as a strong predictor for the occurrence of CAD followed by ADMA, NO, Hcy and ESR as shown in Table 28.

There was also a significant negative correlation observed between NO and age, TG, NTproBNP, ADMA, MPO, Hcy, HbA1c, AER and Fibrinogen (p<0.001). ADMA had significant positive correlation with age, hs-CRP (p<0.05). NTproBNP had significant positive correlation with age, obesity, TG HbA1c, Lp(a), AER and ESR. When we turns to conventional markers Age was found to be positively correlated with TG, NTproBNP, ADMA, MPO, Hcy and negatively with NO levels for the occurrence of CAD. Obesity as a cause of CAD also correlate positively with FS, TG, HDL(decreased), LDL, NTproBNP, Hcy, HbA1c, Lp(a),
hs-CRP and AER. Of lipid profile TG emerged as a strong positive correlate of Age, obesity, NTproBNP, MPO, Hcy, HbA1c, AER and Fibrinogen.

The Table 28 demonstrate that the sensitivity of MPO ≥300 μmol/L at admission for prediction of an adverse outcome of CAD during our study is higher followed by other markers (table-28). Furthermore, specificity for prediction of any adverse outcome of CAD is higher for Hcy followed by hs-CRP and others. Positive predictive value was higher for Hcy followed by Lp(a), ADMA, NO at admission for occurrence of an adverse outcome of CAD during our study. Diagnostic accuracy of Hcy, Lp(a) were highest followed by ADMA and NO levels.

Discussion:--
It provided a detailed compare and contrast the subtle variation existing.
It provided a comparison of present study with published data.

Conclusion:--
Endothelium-derived nitric oxide (NO) is the most potent endogenous vasodilator known, it is released by the endothelium in response to shear stress and plays an important role in flow-mediated vasodilation. Pharmacological inhibition or a genetic deficiency of endothelial NO synthase (NOS) impairs endothelium-dependent vasodilatation and increases vascular resistance and atherogeneity in patients with coronary artery disease with or without diabetes.
Asymmetric dimethylarginine (ADMA) is an endogenous and competitive inhibitor of nitric oxide synthase. Plasma levels of this inhibitor are elevated in patients with atherosclerosis and in those with risk of atherosclerosis. In these patients, plasma ADMA levels are
correlated with the severity of endothelial dysfunction and atherosclerosis. By inhibiting the production of nitric oxide, ADMA may impair blood flow, accelerate atherogenesis, and interfere with angiogenesis. The results of present study concluded that serum ADMA activity measurement may add a significant contribution to CAD diagnosis although NO measurement values the same because it is the main determinant of endothelial dysfunctions.

**Tables and graphs:-**
It included results in tabular form and correlation graphs related to present research.

**Bibliography:-**
It included all references related to present research.

**List of Published Paper: - 6**

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