DISCUSSION

Biochemical investigations help us to understand the chemical pathology of many diseases, in which significant chemical changes occur in the body.

Lipid Profile

High concentrations of serum lipids is an important factor in the pathogenesis of coronary arteriosclerosis (306,311). In general, it is accepted that hypercholesterolemia is one of the three major risk factors for coronary atherosclerosis(312), but evidence concerning the risk from hypertriglyceridemia is conflicting.

In the present study dyslipidemia was observed in all the four groups under study (Table-8). Most derranged lipid profile was found in group I and group IV. It clearly shows that CAD with DM is worse combination for dyslipidemia. Total cholesterol was significantly increased in all the four groups of patients as compared to controls. Higher values were found in group I and IV. HDL was significantly decreased in first three groups, however change in the IVth group was not significant. Triglyceride was significantly increased in patients of group I, II, and IV but no significant variation was observed in group III (i.e. patients without CAD). Highest TG was seen in group IV. LDL was increased significantly in all four groups as compared to controls (Graph 1,2,3,4). The percent variation in different lipid parameters is shown in Graph 5.

Abnormalities in plasma lipoprotein concentrations are commonly observed in diabetic individuals and have a profound impact on the atherosclerotic process (313). Total cholesterol was increased consistently in all the four groups. In general it is accepted that hypercholesterolemia is probably the most important risk factor for development of CAD. Similarly high concentration of other lipids is also an important factor in the pathogenesis of CAD. Though hypertriglyceridemia was observed in all groups with CAD, there was no significant rise in TG in group
III (i.e. patients with DM only). Highest TG was seen in group IV, may be other factors like smoking, obesity, stress etc. contributing for increase in TG. But evidence concerning the risk of CAD from hypertriglyceridemia is conflicting (314).

LDL is known to be atherogenic while HDL is the negative risk factor for atherogenesis and CAD. Hypercholesterolemia along with increase in the LDL observed in all the four groups of patients in the present study with reduced HDL levels has increased the atherogenic process in the patients included in the present study thus increasing the risk of CAD.

Hypertriglyceridaemia is a thrombogenic factor rather than atherogenic factor. In our study as mention earlier decreased HDL cholesterol was observed. It is possible that they primary disturbance responsible for the low HDL may ultimatively lie in triglyceride metabolism and could be a mediator between the basic metabolic defect in the vascular lesion.

Diabetes and dyslipidaemia are independent risk factors for macrovascular disease. It is clearly evident that the combination may be playing a major role in pathogenesis of CAD (315). In the present study, as expected in group II, the level of glycosylated Hb was found to be normal but in other three groups (i.e. I, III and IV ) it was significantly raised (Table No-9, Graph No-6 and 7).

There is an increase in risk of CAD with increased risk for macrovascular disease (316,317). Klein R. (318) in his study showed an increased risk of 11% for each increment of 1% in HbA1c and 10% increase in mortality from ischemic heart disease for an increment of 1% in HbA1c.

Glycosylated proteins can be oxidized to produce free radicals ,which may cause cross linking to produce advance glycosylation end products (AGES).
Graph No 1- Bar chart showing the levels of Total Cholesterol in mg/dl in control subjects and different groups.

Graph No 2- Bar chart showing the levels of HDL in mg/dl in control subjects and different groups.
Graph No 3- Bar chart showing the levels of Triglyceride in mg/dl in control subjects and different groups.

Graph No 4- Bar chart showing the levels of LDL in mg/dl in control subjects and different groups.
Graph 5 - Percent change of cholesterol, HDL, TG, LDL in group I, II, III, IV with respect to control groups.
Accumulation of AGES in the arterial walls may make it more susceptible to a variety of atherogenic influences. Endothelial dysfunction can be related to both, production of AGES and oxidation stress due to elevated glucose levels, causing free radical damage or can be independent and contribute to progression of CHD (315).

Type 2 diabetes, a disease primarily of insulin resistance associated with multiple metabolic derangements like hyperglycemia, dyslipidemia and disorders of coagulation system. These causes are attributed to the vasculopathic state in diabetes mellitus. Patients with diabetes mellitus have increased total cholesterol, LDL & TG in blood, whereas HDL is found to be decreased (44). Similar findings were observed in our study also. Only variation was the inconsistency in the mean TG values. TG was increased in all the groups except group III. Metabolic abnormalities exist in LDL-cholesterol metabolism in diabetes. There is an increase in the proportion of small, dense LDL-cholesterol particles and increased glycation of LDL-cholesterol. Both of them increase atherogenecity. So increased concentration of LDL-cholesterol may be more pathogenic in patients with type 2 DM than in non diabetic patients (319).

Several risk factors for increased levels of LDL in patients with CAD have been identified. Previous studies have suggested that elevated levels of LDL occur in diabetics, smokers and cardiovascular disease (320). It is believed that obesity may not be an independent risk factor for cardiovascular disease but it may increase cardiac risk through metabolic effects, such as dyslipidemia, glucose intolerance, insulin resistance and hypertension, all of which may be associated with increased oxidant stress. HDL-cholesterol may protect LDL- peroxidation but decreased HDL concentration indicates more susceptibility of LDL for oxidation (320) (Fig. 20).
Comparison was made between males and females in our study groups for different parameters like TC, HDL, LDL, TG and glycosylated hemoglobin. No significant variation was found in the levels of different parameters except little elevation in the level of TG in females than males. It was also shown by Sheth T. and Yusuf S. that excess of CAD among overseas Indians has been similar or greater in women than in men (321). The reason for no genderwise variation in lipid profile may be the age group of females in this study, which is between 40 to 60 yrs. Hence the influence of estrogen on lipid metabolism is not significantly different between males and females.

Fig No 20 - Oxidized LDL in Atherogenesis

---Oxidized LDL in atherogenesis. Native LDL becomes trapped in the subendothelial space where it can be oxidized by resident vascular cells, endothelial cells, and macrophages. Oxidized LDL stimulates monocyte chemotaxis, monocyte differentiation and LDL internalization, leading to foam cell formation. Oxidized LDL causes endothelial dysfunction and injury and impairs smooth muscle cell function and vasoreactivity. Antioxidants may inhibit oxidation of LDL and prevent endothelial dysfunction.
Graph No 6- Showing the levels of Glyco-Hb (HbA₁c) (%) in control subjects and different groups

Graph No 7 - Percent change of Glyco-Hb in group I, II, III, IV with respect to controls
Lipoprotein (a)

Lipoprotein (a) is a cholesterol ester-rich lipoprotein composed of an LDL particle and a large hydrophilic glycoprotein, apolipoprotein (a). Lp (a) has recently been categorized as an emerging lipid risk factor for CAD (323). Several control and prospective studies have identified elevated levels of plasma Lp(a) as risk factor for CAD (114,115).

In the present study significant increase was seen in the level of lipoprotein (a) in groups I, II, III and IV compared with control subjects.

Studies in Indian population have shown that Lp(a) levels are significantly higher among coronary artery disease patients as compared to controls (323). Our results are in accordance with these studies (Table No 10, Graph No 8 and 9).

Lp (a) is considered to be an independent level risk factor for premature (324) and multi-vessel CAD (325). Lipoprotein (a) consists of two different components, Apolipoprotein B-100, which binds to LDL ancestors and acts as an atherogenic protein, and Apolipoprotein A, which resembles plasminogen (a zymogen of the coagulation and fibrinolytic system) and competes with the latter for binding to fibrinogen and fibrin monomers, thus acting as prothrombotic agent. Thus, lipoprotein(a) functions as a dual pathogen which is highly atherogenic and is also prothrombotic. Lipoprotein (a) is thus an important molecule in the processes of atherosclerosis as well as thrombosis (326,327). Thrombosis in situ is considered as one of the pathogenic mechanisms in CAD (328). A thrombus may result from injury to the endothelium, abnormal fibrinolysis, enhanced procoagulant activity and/or platelet abnormalities. There are in vivo and in vitro studies which support the theory that lipoprotein (a) has a thrombogenic effect (330-332).

The accumulation of Lp (a) has been well documented in the arterial wall at the site of atherosclerotic lesions (333). Due to its LDL like properties, it accumulates in the atrial valve leading to atherosclerosis.
Lp(a) may induce atherosclerosis in the following manner. The apo(B)-100 which is a component of Lp(a) binds to LDL receptors and acts as atherogenic protein. It also mimicks or blocks plasminogen and competes for binding to fibrinogen, thus acting as a prothrombin agent. Lp(a) inhibits clot lysis (333).

The accumulation of Lp(a) has been well documented in the arterial wall at the site of atherosclerotic lesion. Within intima, Lp(a) can interact with various tissue matrix components including fibrinogen, fibrin and fibronectin. It has also been shown to upregulate the secretion of plasminogen activator inhibitor-1 (PAI-1) and inhibit fibrinolysis. All these effects may be potentiated by concomitant dyslipidemias (334).

Lipoprotein (a) is ten times more atherogenic than LDL. It has dual mechanism of action.

i) Due to its LDL like action it is atherogenic.

ii) Due to its plasminogen like properties, it is thrombogenic.

As mentioned earlier hypertriglyceridemia was observed in our study and hypertriglyceridemia is a thrombogenic factor the thrombic effect of lipoprotein (a) is mimicked by hypertriglyceridemia leading to CAD.
Graph No 8 - Showing the levels of Lipoprotein (a) in (mg/dl) in control subjects and different groups

Fig No 9 - Percent change of Lipoprotein (a) in group I, II, III, IV with respect to controls
Fibrinogen

High plasma fibrinogen level is also a major risk factor for CAD (335). Few workers have observed increased plasma fibrinogen level in CAD. Our results also correlate with these findings. In the present study significant increase in fibrinogen levels was observed in all four groups of patients as compared to control subjects. Significant rise was seen when group IV was compared to group III, however, no significant change was observed between group I, II and III compared to each other (Table No 11, Graph No 10,11).

Most widely recognized as a risk factor in diabetes is fibrinogen, levels of which may be elevated secondary to inflammation. However, fibrinogen is also a precursor of fibrin, which forms the basis of a blood clot and may thus be a factor in the pathogenesis of vascular disease in diabetes.

The increased fibrinogen level observed in the present study may be responsible for enhancing the formation of fibrin clot. In the present study simultaneous increase in Lp(a) is observed and as mentioned earlier, Lp(a) interferes with fibrinolysis. Since it resembles plasminogen and competes with plasminogen for binding to fibrinogen and monomers, thus acting as a prothrombotic agent. It has also been shown to upregulate the secretion of plasminogen activator inhibitor-1 (PAI-1) and inhibit fibrinolysis. This helps in thrombosis. Fibrinogen is also associated with insulin resistance, another cardiovascular risk factor that is mechanistically linked with type 2 diabetes.

Estimation of lipoprotein (a) and fibrinogen levels in DM patients may give an idea about future chances of occurrence of CAD in them, therefore preventive, curative measures for reduction of Lp (a) and fibrinogen can be adapted. Increased incidence of CAD in DM patients can be correlated well with increased levels of lipoprotein (a) and fibrinogen in DM patients.

Many prospective studies have identified an association between plasma fibrinogen concentration and CVD. In another analysis of data from the PHS, those with high fibrinogen levels (after adjusting for LDL and total cholesterol, other
coronary risk factors, and aspirin therapy) had a 2-fold increase in MI risk (336). Data from the Framingham Offspring Population study of 2632 adults suggest a strong relationship between fibrinogen levels and traditional CV risk factors, including age, smoking, diabetes, total cholesterol, high-density lipoprotein cholesterol (HDL-C), and triglycerides (337). Fibrinogen levels were higher in those with CVD than in those without. These data suggest that elevated fibrinogen concentration may be a mechanism by which traditional risk factors exert their effects to contribute to CV events.
Graph No.10 - Showing the levels of Fibrinogen in (mg/dl) in control subjects and different groups.

Fig No. 11 - Percent change of Fibrinogen in group I, II, III, IV with respect to controls
Calcium

Significant associations between serum calcium and blood pressure have been demonstrated in several studies, and it has been hypothesized that serum calcium could play a major role in the development of hypertension (398).

Calcification is closely associated with atheromatous plaque, and is a recognized marker for coronary artery disease (399).

Pathologic studies have demonstrated a strong correlation between the presence of coronary calcium and the amount of atheromatous plaque (174,175).

In the present study sr. calcium was found to be significantly decreased in all the four study groups as compared to control group.

Similarly significant fall in the serum calcium level was observed when Gr. II, III and IV were compared with Gr.I (Table No 12, Graph No 12 and 13).

Abnormalities of calcium metabolism have been described in patients with hypertension (164). Some authors have reported lower concentrations of sr. calcium in the hypertensives than in normotensive subjects. Our results are in accordance with these workers.

Coronary calcification is closely associated with atheromatous plaque and is a recognised marker for CAD. (399) Recent findings indicate that calcification is an active process and calcium is deposited as hydroxyapatite, similar to that in active bone formation (340). It is possible that due to extraction of calcium from the plasma there is lowering of sr. calcium.

Fu. Y., Wang, Touyz, R. M. et al (166,167) has observed significantly reduced serum calcium in hypertensives and the results are in close agreement with that of others who also found a significant decrease in serum calcium in patients with essential hypertension. Reichel et al (341) also reported reduced calcium in males with elevated diastolic blood pressure.
Abnormal cellular ion transport resulting in altered membrane control over intracellular calcium may be related to essential hypertension. Changes in magnesium levels may contribute to altered cell membrane calcium binding in essential hypertension (342).

The free intracellular calcium concentration determines the tension in vascular smooth muscle cells, thereby resulting in peripheral vascular resistance. Calcium has direct effect on peripheral vascular tone (343).

Alterations in intracellular calcium are thought to be involved in the common pathway mediating the secretion and action of many hormones, including the pressor action of catecholamines and angiotension II.

Calcification is not found in normal coronary arteries. The atherosclerotic process starts in the second decade of life, when calcium deposition begins. The calcium deposition increases with age, and with progression of atherosclerotic lesions. Coronary artery calcification is temporarily related to vascular inflammation and the demise of lipid-laden macrophages (344,345). The exact mechanism is unclear, and may be due to the deposition of extracellular calcium from dying cells, or the formation of bone-like structure by calcifying cells (346,347). Since calcium is deposited only in the atherosclerotic plaques and not in the normal vessels, and since atherosclerosis is a diffuse process, a high coronary calcium burden reflects the presence of more extensive coronary atherosclerosis. Hence, higher Coronary calcium scores (CCSs) are associated with the presence of significant CAD.
Graph No. 12 - Showing the levels of Calcium in (mg/dl) in control subjects and different groups

Fig No. 13- Percent change of Calcium in group I, II, III, IV with respect to controls
C-Reactive Protein

The acute-phase response is a major pathophysiologic phenomenon that accompanies acute or chronic inflammation. Acute-phase proteins are defined as proteins whose plasma concentrations increase or decrease by at least 25% during inflammatory states. Measurement of serum levels of acute-phase proteins is useful, because it may reflect the presence and intensity of an inflammatory process. The most extensively studied indicator of the acute-phase response in cardiovascular disease (CVD) is CRP.

In the present study, CRP was found to be significantly increased in all four groups of patients as compared to control group.

Similarly significant rise in serum CRP was observed when Gr. IV was compared with Gr. II and Gr. III (Table No 13, Graph No 14 and 15).

Postulated mechanisms for the association between CRP and the development of CHD include a possible relationship to the extent of coronary atherosclerosis or the extent of inflammation within the atherosclerosis present.

Several lines of evidence suggest a role for CRP in predicting the presence or absence of atherosclerosis. Firstly, CRP is related to standard cardiac risk factors and has been identified within atheroma, particularly co-localized with foam cells. Secondly, inflammation is an essential component in the development of atherosclerosis.

The strong association of inflammatory markers with atherothrombotic coronary events in humans provides clinical evidence, supporting basic data on the role of inflammation in promoting coronary plaque instability (30-32) and thrombotic potential. In previous reports, CRP has been more consistently identified within atheromatous and potentially vulnerable plaques compared with fibrous plaques (348).

The presence of inflammation has been noted since the earliest histologic observations and theories on the development of atherosclerosis. An acute-phase
reactant, hs- CRP plays an important role in the innate immune response and is now recognized as a mediator of atherothrombotic disease. Recent evidence has shown that CRP directly participates in the atherothrombotic process by activating complement, regulating endothelial nitric oxide (NO) synthase expression and NO synthesis, upregulating expression of cellular adhesion molecules, and possibly directly modulating oxidation of LDL (349) (Figure 21).

CRP is known to selectively bind to LDL, particularly the partly degraded LDL found within atherosclerotic plaques and is generally present with it, and activated complement, within such plaques (213). Bound CRP activates complement, is pro-inflammatory and may thus contribute to atherogenesis.

Observations in the present study i.e. Increased level of CRP and LDL supports the possibilities discussed above. CRP bound to LDL activations complement and thus contributes to the atherogenesis which is an inflammatory process.
Fig No. 21- Role of CRP in the development and progression of atherothrombotic processes.

CRP = C reactive protein; LDL = low density lipoprotein; NO = Nitric oxide
Graph No.14 - Showing the levels of CRP in (mg/dl) in control subjects and different groups

Fig No. 15 - Percent change of C- Reactive Protein in group I, II, III, IV with respect to controls
Uric Acid

Uric acid, generated from xanthine by the enzyme xanthine oxidase, is the final product of purine degradation in humans (225).

Serum uric acid (or more correctly, its monoanion uric acid at physiological pH values) has been thought to be, in humans, a metabolically inert end product of purine metabolism without physiological significance (except gouty diathesis). However, serum uric acid has been recently associated with insulin resistance (350,351). Furthermore, in nondiabetic subjects an elevated levels of uric acid has been shown to be an independent predictor of coronary heart disease and total mortality (352-355). Elevated serum uric acid has been found to be closely associated with dyslipidemia, obesity, hypertension, diabetes, smoking and inflammation (356).

The topical role of uric acid and its relation to cardiovascular disease, renal disease, and hypertension is rapidly evolving. Its important role both historically and currently in the clinical clustering phenomenon of the metabolic syndrome, type 2 diabetes mellitus (T2 DM), atheroscleropathy, and non-diabetic atherosclerosis is of great importance.

The association between high serum uric acid and incidence of CAD was reported more than 50 year ago (225) (Gertler et. al. 1951). Since then numerous clinical and epidemiological studies have explored the association more precisely. Such studies confirmed that elevated uric acid was predictor of cardiovascular disease. However, a great controversy arose as to whether elevated uric acid was an independent risk factor for CAD.

In the present study the level of uric acid was significantly increased in all four groups of patients as compared to control group.

Similarly significant rise in the serum uric acid level was observed when Gr. III was compared with Gr. II and Gr.IV was compared with Gr. I and III (Table No 14, Graph No 16,17).
Hyperuricemia could play a role in the pathogenesis of atherosclerosis. Overwhelming evidence suggests that hyperuricemia is linked to obesity (356), hypertension (357), reduced HDL cholesterol (358), hypertriglyceridemia (359), hyperinsulinemia and reduced insulin sensitivity (350,351).

Elevated levels of serum uric acid are due to either an increase in uric acid production or a decrease in its excretion.

Serum uric acid levels have been reported to be inversely related to renal blood flow and directly to renal vascular resistance in both normotensive and hypertensive humans (360,361). Cappuccio et al (362) demonstrated that uric acid levels were independently associated with increased proximal tubular sodium reabsorption in men. This association is strikingly similar to the ability of insulin to promote renal sodium reabsorption that has been suggested to be one of the reasons for the high frequency of hypertension in metabolic syndrome and NIDDM (363). In insulin-resistant states the vasodilatory effect of insulin mediated by nitric oxide is blunted, leading to disturbances in arterial blood flow (364). On the other hand, hyperuricemia has been associated with elevated circulating endothelin levels (365), and one of the major sites of the production of uric acid in the cardiovascular system is the vessel wall and particularly the endothelium (366). It has been demonstrated that cardiac autonomic neuropathy is an independent predictor of stroke in patients with NIDDM (367). Taken together, these findings suggest that high uric acid could also be a marker of sodium retention coupled with impaired hemodynamic reserves and/or disturbed blood flow.

Uric acid is one of the major endogenous water-soluble antioxidants of the body (366). There is accumulating evidence that increased oxidative stress is closely related to diabetes and its vascular complications (368). Thus, high circulating uric acid levels may be an indicator that the body is trying to protect itself from the deleterious effects of free radicals by increasing the production of endogenous antioxidants, eg uric acid. Interestingly, uric acid prevents oxidative modification of endothelial enzymes and preserves the ability of endothelium to mediate vascular dilatation in the face of oxidative stress (366). There is also some
evidence that uric acid may have the direct role in the atherosclerotic process, because human atherosclerotic plaque contains more uric acid than do control arteries (369). Inflammation is one of the features of atherosclerosis, (370) and uric acid crystals may induce inflammatory responses that are reduced by lipoproteins which have an ability to bind uric acid crystals (371). Hyperuricemia via purine metabolism may also promote thrombus formation (372,373).

Increased level of uric acid in the current study might be responsible for the induction of inflammatory process of atherosclerosis as well as to promote thrombus formation. Increased uric acid production may also be an attempt by the body to overcome the oxidative stress associated with diabetes and its vascular complications.
Graph No. 16 - Showing the levels of Uric Acid in (mg/dl) in control subjects and different groups.

Fig No. 17 - Percent change of uric Acid in group I, II, III, IV with respect to controls.
Microalbuminuria

Microalbuminuria (MAU) is a persistent, increased urinary excretion of albumin (374,375). Microalbuminuria is not only an established marker of diabetic nephropathy but (376,377) also has been shown to predict macrovascular complications in non-insulin dependent diabetes (378,379,380).

Proteinuria is the hallmark of renal disease in diabetes and is now recognised as an independent risk factor for cardiovascular disease (381).

In the present study significant Microalbuminuria was observed in all four groups of patients as compared to control.

Similarly significant change was seen when the patient groups were compared with each other. (Table No 16, Graph No 18 and 19).

Diabetes is a chronic condition which poses risk for nephropathy because of increased vascular permeability. Increased Urinary albumin (Microalbuminuria) gives an early signal of incipient diabetic nephropathy. Microalbuminuria is an early feature of excessive capillary leakage (382).

Microalbuminuria is associated with wide spread abnormalities in the vasculature that may manifest as altered vascular reactivity and endothelial dysfunction (383). Stehouwer et al (384) have shown that both endothelial dysfunction and inflammation are involved in the pathogenesis of MAU and poor glycemic controls was associated with increase in markers of endothelial dysfunction and inflammatory activity. They observed that HbA1c was consistently positively associated with longitudinal development of markers of inflammatory activity and endothelial dysfunction.

Microalbuminuria is associated with the accumulation of extracellular matrix in glomeruli and large vessel walls (385). Changes in the quality of the extracellular matrix (386-387) and proliferation of mesagial and myomedial cells (388) have been reported. Similar changes in the extracellular matrix of vessel walls have also been found in atherosclerosis (389). It has been suggested that microalbuminuria is a marker of vascular damage and thus is an early finding in atherosclerosis (390).
The increased microalbuminuria observed in our study points towards the vascular damage in the patients with CAD as well as diabetes mellitus.
Graph No. 18 - Showing the levels of Microalbuminuria in (mg/l) in control subjects and different groups

Fig No. 19 - Percent change of Microalbuminuria in group I, II, III, IV with respect to controls