STUDY OF PLASMA FIBRINOGEN LEVELS IN CORONARY ARTERY DISEASE AS AN INDEPENDENT RISK FACTOR

By

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This is to certify that the ethical committee of Sri Devaraj Urs Medical College, Tamaka, Kolar has unanimously approved, **Dr. GOPINATH B,** student in the Department of General Medicine at Sri Devaraj Urs Medical College, Tamaka, Kolar to take up the dissertation work titled **"STUDY OF PLASMA FIBRINOGEN LEVELS IN CORONARY ARTERY DISEASE AS AN INDEPENDENT RISK FACTOR"** to be submitted to the Sri Devaraj Urs Academy of Higher Education and Research, Tamaka, Kolar.

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LIST OF ABBREVIATIONS

CAD	-	Coronary artery disease
IHD	-	Ischemic heart disease.
CRP	-	C reactive protein

Lp(a)	-	Lipoprotein a
LDL	-	Low density lipoprotein
HDL	-	High density lipoprotein
g/l	-	gram per litre
nm	-	nano meter
SNPs	-	single nucleotide polymorphisms
FGA	-	fibrinogen chains alpha,
FGB	-	fibrinogen chains beta
FGG	-	fibrinogen chains gamma
IL-6	-	interleukin -6
T2DM	-	Type 2 Diabetes Mellitus.
DM	-	diabetes mellitus
HTN	-	hypertension
MI	-	myocardial infarction
AWMI	-	anterior wall myocardial infarction
IWMI	-	inferior wall myocardial infarction
BMI	-	Body mass index
CVD	-	cardio-vascular heart disease
PROCAM	-	Prospective Cardiovascular Munster Study.
ECAT	-	European Concerted Action on Thrombosis and disabilities.
vWF	-	von willebrand factor.
RRs	-	relative risks.
LVMI	-	Left ventricular mass index.
FBS	-	fasting blood sugar

PPBS	-	post prandial blood sugar.
HbA1c	-	Glycated hemoglobin.
mg/dl	-	milligrams per deciliter.
CARDIA	-	Coronary Artery Risk Development in Young Adults.
CAC	-	Coronary artery calcium.
CIMT	-	Carotid intimal-medial thickness.
CT	-	computed tomography.
CC	-	common carotid.
IC	-	internal carotid.
SD	-	standard deviation.

ABSTRACT

BACKGROUND AND OBJECTIVES: Coronary artery disease (CAD) is a major health problem World-wide, and is one of the leading causes of morbidity and mortality. By 2025, cardiovascular mortality on a worldwide scale will likely surpass that of every major disease group. Conventional risk factors both modifiable (like diabetes, hypertension, hyperlipidemia, sedentary life style and smoking) and non-modifiable (like age, male sex and genetic influence) have been identified with cohort of the patients with CAD. Besides these traditional risk factors other novel risk factors like LDL cholesterol, Lipoprotein (a) Lupus anticoagulant, total Homocysteine and High sensitivity C-reactive protein, fibrinogen, D-dimer have also been identified. This study would be undertaken to evaluate the role of plasma fibrinogen as an Independent risk factor in coronary artery disease.

METHODOLOGY: 30 patients who had suffered from acute coronary syndrome 3 months prior to recruitment were included in the study. Patients demographic, anthropometric data were collected, Plasma fibrinogen was analysed using Nephelometry method and other biochemical parameters, were analysed. 30 age and sex matched controls who were free of symptoms of ischemic heart disease were included.

RESULTS: Out of the total 30 cases studied, 14 had anterior wall MI and 16 had inferior wall MI. Mean plasma fibrinogen level amongst cases was 490mg/dl where as in controls, mean fibrinogen levels was 292mg/dl and the difference was statistically significant.

CONCLUSIONS: This study shows that the plasma fibrinogen levels are significantly ($p<0.001$) elevated in patients with coronary artery disease when compared to controls. The conventional risk factors for were not significantly associated with fibrinogen.

KEYWORDS: fibrinogen, coronary artery disease, nephelometry.

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INTRODUCTION

Coronary artery disease (CAD) is a major health problem in the industrialized world as well as in the developing countries and is one of the leading causes of morbidity and mortality. By 2025, cardiovascular mortality on a worldwide scale will likely surpass that of every major disease group, including infection, cancer, and trauma[1,2]

Many conventional risk factors both modifiable (like diabetes, hypertension, hyperlipidemia, sedentary life style and smoking) and non-modiflable (like age, male sex and genetic influence)has been identified with cohort of the patients with CAD. Besides these traditional risk factors other novel risk factors like LDL cholesterol, Lipoprotein (a) Lupus anticoagulant, total Homocysteine and High sensitivity C-reactive protein, fibrinogen, D-dimer have also been identified [3].

Plasma fibrinogen influences platelet aggregation and blood viscosity, interacts with plasminogen binding and, in combination with thrombin, mediates the final step in clot formation and the response to vascular injury. In addition, fibrinogen associates positively with age, obesity, smoking, diabetes, and LDL cholesterol level, and inversely with HDL cholesterol level, alcohol use, physical activity, and exercise level[4].

Fibrinogen, like CRP, is an acute-phase reactant and increases during inflammatory responses.

Given these relationships, it is not surprising that fibrinogen was among the first “novel” risk factors evaluated. Early reports from the Gothenburg, Northwick Park, and Framingham heart studies all found significant positive associations between fibrinogen levels and future risk of cardiovascular events. Since then, a

number of other prospective studies have confirmed these findings and, in recent meta-analyses, there was an approximately linear logarithmic association between usual fibrinogen level and the risk coronary heart disease and stroke [5].

This study would be undertaken to evaluate the role of plasma fibrinogen as an Independent risk factor in coronary artery disease.

OBJECTIVES OF THE STUDY

1) To study the plasma fibrinogen levels in patients with coronary artery disease and controls.

2) To determine the plasma the plasma fibrinogen levels in patients with coronary artery disease as an independent risk factor.

REVIEW OF LITERATURE

FIBRINOGEN

Fibrinogen is a fibrous protein that was first classified with keratin, myosin and epidermin, which was later discovered to be associated with the α-helical coiled structure. It is a glycoprotein normally present in human blood plasma at a concentration of about 2.5g/L, synthesized in hepatocytes, and is essential for hemostasis, wound healing, inflammation, angiogenesis, and other biological functions. It is a soluble macromolecule, but forms a clot or insoluble gel on conversion to fibrin by thrombin. Fibrinogen is also necessary for the aggregation of platelets, and initial steps in hemostasis. In its various functions as a clotting and adhesive protein, the fibrinogen is involved in many intermolecular interactions and has specific binding sites for several proteins and cells. Fibrin clots are dissolved by another series of enzymatic reactions termed the Fibrinolytic System.

There is a dynamic equilibrium between clotting and fibrinolysis, so that the conversion of fibrinogen to fibrin and the dissolution of the clot must be carefully regulated. Any imbalance can result in either loss of blood from hemorrhage or blockage of the flow of blood causing thrombosis. Thrombosis often accompanying atherosclerosis is the most common cause of myocardial infarction and stroke.[6]

FIBRINOGEN STRUCTURE

Fibrinogen molecules are elongated 45 nm structures with nodular regions at each end and in the middle, connected by rod like strands. It consists of two outer D domains, each connected by a coiled-coil segment to its central E domain (Fig. 1). The molecule is comprised of two sets of three polypeptide chains termed *Aα, Bβ, and γ*, which are joined together in the N-terminal E domain by five symmetrical disulfide bridges [7-11]. Each fibrinogen Aa-chain contains an N-terminal fibrinopeptide A (FPA) sequence, cleavage of which by thrombin initiates fibrin assembly [12–14] by exposing a polymerization site termed EA. Each EA-site combines with a constitutive complementary-binding pocket (Da) in the D domain of neighboring molecules [15] [16] [17]. The initial EA : Da associations cause fibrin molecules to align in a staggered overlapping end to middle domain arrangement to form double-stranded twisting fibrils [18–21] (Fig. 2). Fibrils also undergo lateral associations to create multi-stranded fibers [22-23]

Two types of branch junctions occur in fibrin networks [24]. The first occurs when a double-stranded fibril converges laterally with another fibril to form a four-stranded fibril, a so called ‘bilateral’ junction. Lateral convergence of additional fibrils results in multi-stranded versions of this structure. The second type of branch junction, termed ‘equilateral’, is formed by convergent interactions among three fibrin molecules that give rise to three double-stranded fibrils. Equilateral junctions form with greater frequency when fibrinopeptide cleavage is relatively slow [25], and under such conditions, the networks are more branched and the matrix is tighter (i.e. less porous) than those formed at high levels of thrombin.

Genetic factors make an important contribution to plasma fibrinogen levels in humans. Human fibrinogen is the product of three closely linked genes located on the long arm of chromosome 4, each specifying the primary structure of the three polypeptide chains.[26].

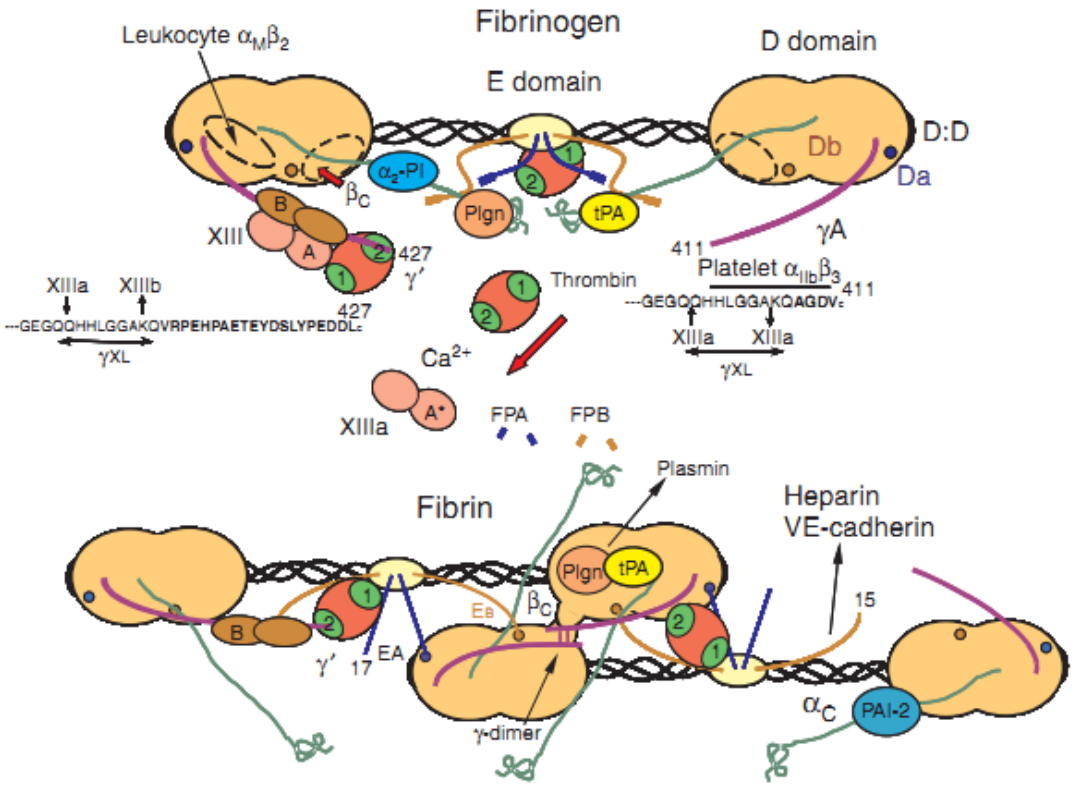


Figure 1: Schematic diagram of fibrinogen structure, its conversion to fibrin, and the thrombin-mediated conversion of native factor XIII to XIIIa. Binding sites for proteins, enzymes, receptors, and other molecules that participate in fibrin(ogen) functions are illustrated.

GENETIC FACTORS INFLUENCING FIBRINOGEN

Genetic factors are estimated to contribute to nearly 50% of the total variability in fibrinogen levels. Several polymorphisms have been identified in the genes encoding the 3 pairs of fibrinogen polypeptide chains, α, ß, and γ; however, because the synthesis of the ß-chain is rate-limiting in vitro, most studies have focused on this gene. The main ß-chain variants include the Arg448Lys, B*cl*I, -148C/T, -455G/A, and -854G/A polymorphisms. The promoter polymorphisms are in strong linkage disequilibrium with each other. The -455G/A and -854G/A substitutions are the most physiologically relevant, because the respective alleles have distinct nuclear protein-binding properties, and reporter gene studies in HepG2 cells showed an increased rate of basal transcription in the less common -455A and -854A alleles.[27,28]. Of the ß-chain polymorphisms, the -455G/A has been the most extensively studied clinically. The -455AA genotype is present in 10% to 20% of the population and is correlated with fibrinogen levels that are 10% higher than in individuals with the GG genotype. Nevertheless, the relation between the -455G/A variant and the risk of arterial thrombotic disease is controversial, with some case-control studies, including the Etude Cas-Temoins sur l'Infarctus du Myocarde (ECTIM) study,[29] indicating an association, while other large studies reported none[30]. In a pooled analysis of inherited hemostatic risk factors and the risk of acute MI, homozygosity for the fibrinogen -455A allele was significantly though marginally associated with a decreased risk of MI (odds ratio [OR], 0.66; 95% confidence interval [CI], 0.44 to 0.99).[31] In addition, the -455A allele has been associated with the progression of atheroma.[26] In a recent cohort of elderly patients with stroke, the presence of the -455A allele was associated with a 2.5-fold increase in risk of multiple lacunar infarcts but not with large-artery strokes; the authors suggested that elevated

fibrinogen levels might predispose to the development of thrombosis primarily in small arteries[32].(look into genetic determinants of arterial thrombosis for references).

The AIRGENE study [33] was designed to investigate whether single nucleotide polymorphisms (SNPs) and haplotypes of the fibrinogen gene-cluster (fibrinogen chains alpha [FGA], beta [FGB], and gamma [FGG]) could explain the inter and intra-individual variability of fibrinogen levels in patients with atherosclerosis. This study also searched for genetic determinants affecting the responses of fibrinogen genes to proinflammatory stimulation. In this study, 895 survivors of myocardial infarction from 5 European cities were followed prospectively for 6 to 8 months, and plasma fibrinogen, interleukin (IL)-6, and C-reactive protein levels were determined monthly. 21 SNPs and the corresponding haplotypes in the 3 fibrinogen genes were analyzed. Eight SNPs in FGA and FGB were significantly associated with fibrinogen levels. Similarly, 2 different haplotypes in FGA and 3 in FGB were also associated with mean fibrinogen levels. The IL-6 levels had a significant impact on the associations between SNPs/haplotypes in FGA/FGB and fibrinogen levels. This study also identified SNPs and haplotypes in FGA and FGB with strong impact on the intra-individual variability of fibrinogen during the follow-up period. This study identified common SNPs and haplotypes on FGA/FGB genes, explaining the inter-individual and intra-individual variability of fibrinogen levels, in patients with a history of myocardial infarction. This study also identified for the first time, SNPs/haplotypes on FGA/FGB whose effects on fibrinogen expression are modified by the underlying IL-6 levels. These findings may have an impact on risk stratification and the design of genetically guided therapeutic approaches in patients with advanced atherosclerosis.

METHODS TO ASSESS SERUM LEVELS OF FIBRINOGEN

The following are the methods to assess serum fibrinogen

1) The kinetic fibrinogen assay
2) The Von Clauss method
3) The Thrombin-coagulable fibrinogen method
4) Nephelometry

1. **The kinetic Fibrinogen assay.** The determination is based on addition of a commercially prepared thromboplastin reagent to undiluted plasma, followed by the measurement of turbidity increase. By using the clotting factors provided by the native plasma, the KFA method very closely approximates the *in vivo* reaction between human thrombin and fibrinogen. For quantitative determination, the microsample coagulation analyser is used and this instrument electronically transforms results to calculate the first derivative of the rate of turbidity increase change that is proportional to fibrinogen concentration in plasma. The required reagents, thromboplastin preparation, normal human control plasma and fibrinogen calibration plasmas to establish the reference curve. Each plasma specimen is measured in sictuplicate and the mean value of fibrinogen concentration is calculated.

2. **The von Clauss method.** The procedure is a clotting rate method in which addition of a very high bovine thrombin concentration to a 20-fold diluted plasma overcomes endogenous or exogenous inhibitor and renders the logarithm of the clotting time directly proportional to the logarithm fibrinogen concentration [34]. The von clauss method requires a calibrated standard to

establish the exact parameters of determination. This is one of the most commonly used assays. Each plasma specimen is measured in triplicate and the mean value of fibrinogen concentration is calculated.

3. **The Thrombin–coagulable fibrinogen method** is direct assay that does not require any standards. The principle of this method is to isolate thrombin-coagulable material from citrated plasma with exclusion of factor XIIIa-mediated cross linking. Once the clot is formed, it is washed extensively and dissolved in alkaline urea for spectrophotometric measurement of fibrin concentration. An absorption coefficient of 1.587 is used for a fibrinogen solution at 1mg/ml. the protocol recommended by the WHO [35] an amalgam from two methods described by Jacobsson [36] and Blomback and colleagues [37] is particularly accurate version of the clot collection and quantification. Each plasma specimen is measured in triplicate and the mean value of fibrinogen concentration calculated.

4. **Nephelometric procedure:** In principle, the test consists of heating at 56 C^0 for 20 min either 0.30 ml of plasma diluted with 9.7 ml reagent (if normal or supra-snormal concentrations are anticipated), or 0.30 ml of plasma diluted with 4.7 ml of reagent (if hypofibrinogenemia is expected). The denatured fibrinogen suspension was measured either nephelometrically against an unheated sample with the nephelometer, or turbidimetrically at 600 nm with the spectrophotometer. The manufacturer's standardization factor for the nephelometer was derived (by D.E.R.M.) from fibrinogen assays of 20 human plasma samples by a method involving sodium sulfite precipitation and subsequent Kjeldahl nitrogen determinations. The resulting "Hach" factor was checked independently (by E.W.R.) by comparing the nephelometric results of

50 samples with those obtained by the “reference” clottable-protein procedure of Grannis.

Comparison of different methods for measurement of fibrinogen concentration in human plasma was reprted by Rossi and collagues [38] Parlaretti and associates [39] and Bovill and colleagues [40]. General conclusions derived from these studies seem to favor the von Clauss method for routine determination of fibrinogen in clinical laboratories. Other photo optical methods (ACL, for example) were shown to correlate with the von Clauss method.

Other methods to estimate fibrinogen:

1) Nephelometry
2) Quantitative method modified from Ratnoff and Menzie
3) Heat precipitation method
4) Salt precipitation method
5) Immunological methods.

Variations in the normal serum fibrinogen levels

Plasma fibrinogen level is dependent upon both genetic and environmental factors. Genetic factors influencing fibrinogen levels has been explained above.

Extrinsic factors

There is evidence that plasma fibrinogen level and its associated cardiovascular risk may be dependent upon an interaction between environmental and intrinsic (genetic) factors rather than just the genetic.

Fibrinogen is an acute phase reactant and its production may increase as a result of various essentially nonspecific stimuli. Indirect evidence exists that the plasma levels of FDP, possibly acting as a feedback control may constitute an important regulator of the rate of fibrinogen synthesis. But the hypothesis has been questioned. Hyperfibrinogenemia induced by pyrogens and other bacterial extracts may be mediated through an effect on leucocytes.

Numerous interaction between fibrinogen and other variables have been described in cross- sectional epidemiologic studies. The largest such Study (n= 15803) showed fibrinogen to be 0.2 g/L higher in blacks than in whites. [41]. It also confirmed that women had higher value than men. Fibrinogen increased with age, smoking , body size ,diabetes, LDL, Lp(a), Leucocyte count and menopause. It is decreased with ethanol intake, exercise, HDL, female hormonal use. There is a continuous positive correlation between most lipid parameters and fibrinogen. [41]. Fibrinogen levels are elevated in patients with type II hyperlipoprotienemia [42] and familial hypercholesterolemia.

Smoking is the strongest known determinant of plasma fibrinogen levels in healthy persons. The effect is dose related and reversible on cessation of smoking. [43,44]. Plasma viscosity was elevated in male but not female smokers[45]. The Framingham study provided detailed analysis of the inter relation of fibrinogen with smoking and cardiovascular disease and estimated that 50% of the cardiovascular harm caused by chronic smoking is mediated through its effect of increasing fibrinogen. [46,47].

Fibrinogen levels are higher in patients with essential hypertension than in normotensive controls [48].

Fibrinogen levels are higher in diabetic patients [49]. Patients with micro vascular complications have higher fibrinogen levels than without it. The Framingham study proved a correlation between blood sugar levels and fibrinogen [49]. Fasting glucose also correlates with fibrinogen in non-diabetic persons [41]. In diabetics with albuminuria fibrinogen is higher than in patients without this complication [50]. Finally fibrinogen has been shown to be an independent predictor of vascular complications in type II diabetes.

TABLE 1: Influence of socio-demographic features on normal fibrinogen levels.

High fibrinogen	**Low fibrinogen**
Black Race	Caucasians
Men	Women
Advanced age	Young age
Smoking	Alcohol
Obesity	Weight reduction
Elevated cholesterol	
Menopause	Post-menopausal hormonal substitution
Low socio economic class	
Physical inactivity	Regular physical activity
Oral contraceptive use	
Stress	
Elevated leucocyte count	

CLINICAL STUDIES

In a major meta-analysis [51], which set out to assess the relationships of fibrinogen levels with risk of major vascular and with risk of nonvascular outcomes based on individual participant data, 31 studies were included. This meta-analysis differs from previous analyses in several ways that should increase its reliability and scientific value. This meta-analysis includes 154 211 participants in 31 prospective studies with available individual records, no known history of previous CHD (ie, MI or angina, which was defined in each study) or stroke at baseline, fibrinogen levels of 5.62 g/L or lower, and any nonfatal CHD and stroke events or any deaths recorded for individuals aged 40 years or older. Of these participants, 115 658 (75%) were from Europe, 27 758 (18%) were from North America, and 10 795 (7%) were from Japan. During 1.38million person-years of follow-up (mean)[SD], 8.9 [4.9] years to first event or death), there were 4681 nonfatal MIs,2263 nonfatal strokes, and 13 210 deaths comprising 2437 deaths attributed to CHD, 512 to stroke, 992 to other vascular causes (including 175 due to aortic aneurysm, 125 to heart failure, and 88 to acute pulmonary heart disease), 8007 to nonvascular causes (of which 4856 were due to cancer), and 1262 to unknown causes. There were generally strong and highly significant positive associations between plasma fibrinogen levels and values of several established risk factors at baseline, such as age, cigarette smoking, serum levels of total and low-density lipoprotein cholesterol and triglycerides, blood pressure, body mass index, and history of diabetes (and, in the subset with data available on inflammatory factors, with leukocyte count and C-reactive protein values). There also were highly significant inverse associations between plasma fibrinogen level and male sex, alcohol consumption, and serum levels of high-density lipoprotein cholesterol and albumin. This study further showed that the associations of

fibrinogen level with CVD or with stroke do not differ substantially by baseline levels of established risk factors such as sex, smoking, blood pressure, serum lipid levels, or several features of study design. This analysis provides the most comprehensive and detailed description, to date, of the observational epidemiological associations between plasma fibrinogen level and a range of different chronic disease outcomes and demonstrates moderately strong associations in a wide range of circumstances.

A meta-analysis [52] evaluated the possibility of fibrinogen as a cardiovascular risk factor. A computerized literature search (1980 to 1992) identified all published epidemiologic studies on fibrinogen and cardiovascular disease. Six prospective epidemiologic studies were included in a meta-analysis (one study was excluded because the study population was non-representative). Clinical papers were reviewed separately for other evidence of causation. The correlation of fibrinogen levels on the subsequent incidence of myocardial infarction, stroke, and peripheral arterial occlusive disease was assessed and the causality of the association was analyzed. Calculations were made to examine fibrinogen level (in tertiles) versus cardiovascular risk. Odds ratios of high versus low tertile were computed. All prospective studies showed that fibrinogen was associated with subsequent myocardial infarction or stroke. A total of 92,147 person-years was covered by these investigations. Odds ratios varied between 1.8 (95% CI, 1.2 to 2.5) in the Framingham and 4.1 (CI, 2.3 to 6.9) in the GRIPS study, with a summary odds ratio of 2.3 (CI, 1.9 to 2.8). Associations existed between fibrinogen and other cardiovascular risk factors, but after multivariate analysis, only the association between fibrinogen and cardiovascular events remained. Plasma fibrinogen was associated with ‘true’ risk factors such as diabetes, hypertension and hypercholesterolemia in the studies included in this meta-analysis. The majority of the preconditions for causality were fulfilled, indicating that

fibrinogen is pathophysiologically related to cardiovascular events. This study concluded that Fibrinogen can be considered a major cardiovascular risk factor and suggested that future studies of cardiovascular morbidity and death should include this variable.

The Northwick Park Heart Study (NPHS) [53] has investigated the thrombotic component of ischaemic heart disease (IHD) by the inclusion of measures of haemostatic function. Among 1511 white men aged between 40 and 64 at the time of recruitment, 109 subsequently experienced first major events of IHD High levels of factor VII coagulant activity and of plasma fibrinogen were associated with increased risk, especially for events occurring within 5 years of recruitment. These associations seemed to be stronger than for cholesterol, elevations of one standard deviation in factor VII activity, fibrinogen and cholesterol being associated with increases in the risk of an episode of IHD within 5 years of 62%, 84%, and 43% respectively. Multiple regression analyses indicated independent associations between each of the clotting factor measures and IHD but not between the blood cholesterol level and IHD incidence. The risk of IHD in those with high fibrinogen levels was greater in younger than in older men. Much of the association between smoking and IHD may be mediated through the plasma fibrinogen level. The biochemical disturbance leading to IHD may lie at least as much in the coagulation system as in the metabolism of cholesterol.

The Scottish Heart health Study [54] was designed ,to determine the relations of plasma fibrinogen to family history of premature heart disease, personal history of hypertension, diabetes, stroke, coronary heart disease, and to presence of intermittent claudication. It was a random population survey across 22 local government districts in Scotland. The participants were 10,359 men and women aged 40 to 59 years

Plasma fibrinogen was measured in 8824. The main outcome measure was Plasma fibrinogen concentration. The study found that persons with a family history of heart disease or a personal history of high blood pressure, diabetes, stroke, or presence of intermittent claudication all had higher plasma fibrinogen concentrations than those without. When compared with participants without cardiovascular or related disease (men: 2.27 (SE= 0.01) g/l. n=3367; women 2.34(0.01)g/l, n=3096, predefined cases of either myocardial infarction{men:2.51(0.02)g/l, n=248; women:2.63(0.04)g/l, n=72} or angina {men: 2.45(0.02)g/l, n=394; women: 2.50(0.02) g/l, n=398} had significantly higher plasma fibrinogen concentrations($p< 0.001$). After adjustment for 10 other coronary risk factors, there was a noticeable linear trend the odds ratios for myoardiaI infarction across all quartiles(quarters) of plasma fibrinogen concentrations in both sexes. Similarly, the risk of angina increased linearly with increasing fibrinogen concentrations, although the test for a linear trend was NS among women. The study concluded that this large population study concluded that plasma fibrinogen is not only a risk factor for coronary heart disease and stroke, but it is also raised with family history of premature heart disease and with personal history of premature heart disease and with personal history of hypertension, diabetes and presence of intermittent claudication.

Gothenburg study [55]. In a random sample of 792 men aged 54 years, MI occurred in 92 men, stroke in 37, and death from causes other than MI or stroke in 60 during 13.5 years of follow-up. Plasma fibrinogen was an independent risk factor for MI and stroke on univariate analysis. On multivariate analysis, plasma fibrinogen was still statistically significant for stroke risk.

In the Leigh General Practice Study, [56] 505 men aged 40–69 years and free from IHD, diabetes and hypertension were recruited from one general practice in the

UK. After a mean follow-up of 7.3 years, 40 cases of MI occurred. On multivariate analysis, plasma fibrinogen proved to be the strongest predictor of adverse cardiovascular events, with an OR of 21:1 when those with high levels (> 3.5 g/l) were compared to those with low levels (< 2.9 g/l) of fibrinogen.

In the Framingham Study, [57, 58] the risk of developing cardiovascular disease was significantly related to plasma fibrinogen levels. In both sexes, cardiovascular and stroke risk increased progressively in relation to antecedent fibrinogen values over the 1.8–4.5 g/l range. As in NPHS, the influence of plasma fibrinogen on cardiovascular risk was much more pronounced in younger men. The impact of plasma fibrinogen levels on cardiovascular disease was comparable with the major risk factors, such as blood pressure, haematocrit, adiposity, cigarette smoking and diabetes; and was still an independent predictor of coronary artery disease on multivariate analysis.

In the Munster Heart Study (Prospective Cardiovascular Munster Study, (PROCAM), [59] plasma fibrinogen factor VIIc, blood pressure, and lipid parameters were measured in 2781 healthy men aged 40–65 years. After 8 years of follow-up, 130 coronary events were observed, and the mean plasma fibrinogen level of the 'event group' exceeded that of the non-event group by 0.32 g/l. The incidence of coronary events among men within the upper tertile of plasma fibrinogen concentration was threefold higher than among men within the lower tertile. When fibrinogen and LDL concentration were considered together, there was a graded and dramatic eightfold increase in 8-year risk among men with both fibrinogen and LDL cholesterol in the higher tertiles, when compared to men with both of these parameters in the lower tertile.

The Caerphilly and Speedwell collaborative heart disease studies [60], these two studies were based on a combined cohort of 4860 middle-aged men from the general population. After a follow-up of 5.1 years in the Caerphilly study and 3.2 years in the Speedwell study, 251 major IHD events occurred. The age-adjusted relative odds of IHD for men in the top 20% of the distribution compared with the bottom 20% were 4.1 for fibrinogen, 4.5 for viscosity, and 3.2 for white blood cell count. Multivariate analysis showed that white blood cell count, fibrinogen and viscosity were independent risk factors for IHD.

European Concerted Action on Thrombosis and disabilities (ECAT) study [61] plasma fibrinogen was a strong and independent risk factor for MI and sudden death, particularly in patients with pre-existing coronary artery disease, along with plasma von Willebrand factor (vWF) antigen (a marker of endothelial damage), and tissue plasminogen activator antigen (a marker of thrombolytic activity). In patients with coronary artery disease, the relationship of plasma fibrinogen levels to the incidence of acute coronary syndromes was stronger than that of low-density lipoprotein cholesterol. Fibrinogen (RR 1.31, 95%CI 1.07–1.61) had a stronger association with future coronary events than either vWF antigen (RR 1.24, 95%CI 1.00–1.53) or t-PA antigen (RR 1.29, 95%CI 1.04–1.60).

At baseline in the Cardiovascular Health Study [62] (5888 white and African American men and women; aged ≥65 years), fibrinogen, factor VIII, and factor VII were measured. Sex-stratified stepwise Cox survival analysis to determine relative risks (RRs) for CVD events and all-cause mortality (up to 5 years of follow-up), both unadjusted and adjusted for CVD risk factors and subclinical CVD. After adjustment, comparing the fifth quintile to the first, fibrinogen was significantly associated in men

with coronary heart disease events (RR=2.1) and stroke or transient ischemic attack (RR=1.3), and also with mortality within 2.5 years of follow-up (RR=5.8) and later (RR=1.7). Factor VIII was significantly associated in men with coronary heart disease events (RR=1.5) and mortality (RR=1.8), and in women with stroke/transient ischemic attack (RR=1.4). For both factors, values were higher in those who died, whether causes were CVD-related or non–CVD-related, but highest in CVD death. Factor VII exhibited associations with incident angina (RR=1.44) in men and with death in women (RR, middle quintile compared with first=0.66). However, in general, factor VII was not consistently associated with CVD events in this population. We conclude that, if confirmed in other studies, the measurement of fibrinogen and/or factor VIII may help identify older individuals at higher risk for CVD events and mortality.

In Edinburg artery study [63], Blood and plasma viscosity, hematocrit, fibrinogen, urinary fibrinopeptide A, plasma leukocyte elastase, and uric acid were measured in a random sample of 1,581 men and women aged 55-74 years in Edinburgh, Scotland, and related to peripheral arterial stenosis (ankle-brachial systolic pressure index, ABPI) and to lower limb ischemia (intermittent claudication and reactive hyperemia test). Each variable (except fibrinopeptide A) was significantly related to prevalent symptomatic and asymptomatic peripheral arterial disease. On multivariate analysis, blood viscosity ($p<0.05$) and fibrinogen ($p<0.01$) were independently associated with peripheral arterial narrowing (ABPI); a positive interaction was found between fibrinogen and smoking in the association with ABPI. This study concluded that Blood rheological factors and leukocyte activation as well as arterial narrowing are associated with lower limb ischemia in the general population and may be implicated in its pathogenesis.

In a prospective Japanese study [64], plasma fibrinogen was studied in relation to coronary heart disease. The subjects were population-based samples of 7,261 men aged 30 years from Osaka city and 4,973 men and women aged 20 years and over who lived in Yao City in Osaka Prefecture, Japan. Their age range was 21– 89 years, and only 3 percent of the participants were under age 34 or over age 76 years. The survey was conducted between 1990 and 1996. A total of 257 persons with a history of either coronary heart disease (n= 179) or stroke (n= 72) or both (n= 6) were excluded. The remaining 11,977 persons were followed to ascertain the incidence of coronary heart disease through March 31, 1998. The plasma was separated from patient's blood and stored at –70˚C for 2 weeks until the measurement. Fibrinogen was measured by the clotting assay of Clauss. The mean value of plasma fibrinogen was 267 mg/dl (standard deviation (SD), 59) in this cohort. During the 4.8 years of follow-up, 41 incident coronary heart disease events occurred (21 definite myocardial infarctions, 11 suspect myocardial infarctions, and nine angina pectoris. This study found that plasma fibrinogen was higher in Japanese men and women who developed coronary heart disease than in persons free of the disease. This relation remained statistically significant even after adjustment for major covariates, such as age, sex, smoking status, and serum total cholesterol. The positive association was similarly observed in younger and older age groups and was not confined to the early follow-up periods. This study was the first to propose that raised plasma fibrinogen is a significant predictor of increased risk of coronary heart disease in Japanese individuals.

In one Indian study [65], interrelation of Fibrinogen, Lp (a) and LVMI in Type II diabetes patients with or without nephropathy was studied. 100 patients of Type II diabetes (age 45–65 years) visiting Endocrinology OPD of DMC&H were included in

the study. Patients with previous history of CVD, hypertension, smokers were excluded from the study. Patients were divided into two groups of 50 each. Group I: without MAU and Group II: with MAU (30Male and 20 females in each group). Overnight fasting blood and urine sample was collected for biochemical investigations. FBS, HbA1c, Lp (a) and MAU were analyzed on auto-analyzer Hitachi 911(Roche). Fibrinogen was estimated by Clauss Method using Sigma diagnostic kits. LVM was calculated by echocardiography using formula from Devereux and Riechek. There was no significant difference in age of both the group patients. FBS of all the patients was [140 mg/dl and HbA1c was [7.0 g% showing poor glycemic control. Diabetic patients with MAU (group II) had significantly higher levels of LVMI, Fibrinogen and Lp(a) as compared to diabetic patients without MAU. Group II patients had positive correlation between Lp(a) and LVMI.

The Coronary Artery Risk Development in Young Adults (CARDIA) [66] study, a multi-center longitudinal study designed to investigate the evolution of CVD risk factors and sub-clinical atherosclerosis. The initial cohort included 5115 black and white adults aged 18–30 years at baseline (1985–1986) were recruited. Age, race, gender and education were roughly balanced by the sample design. To date, six follow-up examinations have been completed at years 2, 5, 7, 10, 15 and 20. In 1992 and 1993, as part of the year 7 CARDIA examination (termed baseline or Y7 in this article), fibrinogen was measured along with other clinical, demographic and health variables. Fibrinogen was again measured during the year 20 CARDIA examination in 2005 and 2006 (termed follow-up or Y20 in this article). Of the 4024 participants returning for the Y7 examination, 808 did not return at Y20 and 40 of those who did return did not have Coronary artery calcium (CAC) and carotid intimal-medial thickness (CIMT) studies. Fibrinogen measurements were not obtained in 168, and

39 were excluded because of pregnancy, heart disease or missing data. The final sample for longitudinal analyses included 2969 participants (517 black men, 788 black women, 816 white men and 848 white women). Of the 3048 persons returning at Y20, 104 were pregnant, persons with heart disease (heart attack, angina, stroke and TIA), or had missing covariates. Forty did not have CAC and CIMT examinations, and fibrinogen was not assayed in 72, leaving 2832 for the cross-sectional analyses. CAC was measured by computerized tomography (CT) of the chest. High-resolution B-mode ultrasonography was used to capture images of the bilateral common carotid (CC) and internal carotid (IC) arteries using a Logiq 700 ultrasound machine. This study confirms one observation that an elevated fibrinogen level in youth is independently associated with subclinical cardiovascular disease in middle-age. However, we now find that the associations of fibrinogen with subclinical disease are attenuated over time, suggesting that fibrinogen might be important when atherosclerosis is developing, but that its predictive role declines when the disease is established. By the fourth decade, factors known to decrease fibrinogen synthesis, such as age and smoking cessation, could modify the association. Fibrinogen was more strongly associated with CAC than with CIMT. This study showed that fibrinogen measured at age 25–37 is more closely associated with atherosclerosis detected at age 38–50 than is fibrinogen measured at the latter age. In these older adults, other cardiovascular risk factors such as obesity and current smoking weaken the associations of fibrinogen with subclinical atherosclerosis.

A meta-analysis study [67], involving 9 community based prospective cohort study, analyzed the associations of fibrinogen levels with cardiovascular disease (CVD) risks in people with and without diabetes, and quantified the value of adding fibrinogen to the established predictive algorithms for CVD. In this study, individual

participant data of English and Scottish Health Survey cohorts to investigate: (1) the association, if any, between fibrinogen and mortality from all-causes and cardiovascular disease in people with and without diabetes; (2) whether measurement of fibrinogen improves discrimination of these outcomes relative to risk algorithms containing established risk factors in people with and without diabetes. Participants were 33,091 individuals (17,965 female) taken from nine prospective British studies comprising either Scottish Health Surveys (six cohorts: 1994, 1998, 1999, 2000, 2003 and 2004) and the Health Surveys for England (three cohorts: 1995, 1998 and 2003). Duration of mortality surveillance was between 0 and 169 months (median 116). Study members were visited twice in their homes. During the first of these visits socio-economic and demographic profile of the patients were collected, interviewers also collected information about physician-diagnosed CVD (stroke, ischaemic heart disease, angina symptoms), other medical conditions (hypertension and diabetes) and anti-hypertensive medication (beta-blockers, angiotensin converting enzyme-inhibitors, diuretics, calcium blockers). During the second visit, physical examination data and blood samples for fibrinogen assessment were collected. Of the 33,091 participants included in the analytical sample, 1006 (3.04%) had diabetes at baseline. In conclusion, fibrinogen is positively and continuously associated with CVD and all-cause mortality. These associations are similar in people with diabetes and people without. Knowledge of information about fibrinogen significantly improves predictive accuracy of models based upon established risk factors similarly in people with diabetes and those without. Such improvement, however, is only modest and, alone, fibrinogen will achieve little in clinically reclassifying patients already risk stratified based on established risk factors.

Epidemiological evidence suggests that fibrinogen and CRP are associated with coronary heart disease risk. High CRP in Indigenous Australians has been reported in previous studies including our 'Diabetes and Related diseases in Urban Indigenous population in Darwin region' (DRUID) Study [68]. We studied levels of fibrinogen and its cross-sectional relationship with traditional and non-traditional cardiovascular risk factors in an urban Indigenous Australian cohort. Fibrinogen data were available from 287 males and 628 females (aged ≥ 15 years) from the DRUID study. Analysis was performed for associations with the following risk factors: diabetes, HbA1c, age, BMI, waist circumference, waist-hip ratio, total cholesterol, triglyceride, HDL cholesterol, C-reactive protein, homocysteine, blood pressure, heart rate, urine ACR, smoking status, alcohol abstinence. Fibrinogen generally increased with age in both genders; levels by age group were higher than those previously reported in other populations, including Native Americans. Fibrinogen was higher in those with than without diabetes (4.24 vs 3.56 g/L, $p < 0.001$). After adjusting for age and sex, the following were significantly associated with fibrinogen: BMI, waist, waist-hip ratio, systolic blood pressure, heart rate, fasting triglycerides, HDL cholesterol, HbA1c, CRP, ACR and alcohol abstinence. On multivariate regression (age and sex-adjusted) CRP and HbA1c were significant independent predictors of fibrinogen, explaining 27% of its variance; CRP alone explained 25% of fibrinogen variance. On factor analysis, both CRP and fibrinogen clustered with obesity in women (this factor explained 20% of variance); but in men, CRP clustered with obesity (factor explained 18% of variance) whilst fibrinogen clustered with HbA1c and urine ACR (factor explained 13% of variance). Fibrinogen is associated with traditional and non-traditional cardiovascular risk factors in this urban Indigenous cohort and may be a useful biomarker of CVD in this high-risk population. The

apparent different associations of fibrinogen with cardiovascular disease risk markers in men and women should be explored further.

METHODOLOGY

SOURCE OF DATA: Patients were recruited from medicine opd visiting to SRI R L JALAPPA HOSPITAL AND RESEARCH CENTRE, TAMAKA, KOLAR, from 1st February 2011 to 31st January 2012.

STUDY DESIGN: Hospital based prospective study.

METHOD OF COLLECTION OF DATA.

1) 30 Patients who had suffered from an acute coronary syndrome 3 months prior to recruitment were included in the study presenting at SRI R L JALAPPA HOSPITAL AND RESEARCH CENTRE attached to SRI DEVRAJ URS MEDICAL COLLEGE.

2) 30 Controls were studied who were matched for age and gender. Controls were free of symptoms of IHD, had no history of previous hospitalization for the same and had no ECG evidence of IHD.

METHODOLOGY:

Each patient enrolled for the study, was evaluated for detailed history regarding risk factors for CAD such as hypertension, diabetes, smoking and alcohol consumption was taken.

Thorough general and systemic examination was carried out, anthropometric measurements like height, weight was taken and BMI was calculated. In addition blood samples for FBS, Lipid profile also collected. Diabetes was diagnosed by American Diabetes Association criteria. Hypertension was diagnosed using JNC VII

criteria. Smoking was recorded in terms of cigarette pack years. MI and Unstable angina was defined according to American heart associations

In addition to routine investigations, plasma fibrinogen levels were estimated in cases and controls. The blood samples were collected in citrate vacutainers, the samples collected were centrifuged to make RBC poor and platelet poor. The plasma thus separated was sent for analysis. The method used for fibrinogen assessment is NEPHELOMETRY METHOD. In principle, the test consists of heating at 56 C^0 for 20 min either 0.30 ml of plasma diluted with 9.7 ml reagent (if normal or supra-snormal concentrations are anticipated), or 0.30 ml of plasma diluted with 4.7 ml of reagent (if hypofibrinogenemia is expected). The denatured fibrinogen suspension was measured either nephelometrically against an unheated sample with the nephelometer, or turbidimetrically at 600 nm with the spectrophotometer.

INCLUSION CRITERIA

1) 30 Patients with old and/or stable ischemic heart disease.
2) TMT positive cases.
3) Angiographlcally demonstrated if available.

EXCLUSION CRITERIA FOR CASES.

1) Acute coronary syndrome with in past 3 months.
2) Patients with known liver disorders.
3) Patients with acute illness or fever.

4) Patients using oral contraceptives.

CONTROLS

1) 30 controls will be included with following parameters
2) No history suggestive of myocardial infarction or angina.
3) No ECG evidence of IHD.
4) Age and gender matched subjects.

PARAMETERS

THE FOLLOWING PARAMETERS WERE TESTED IN ALL CASES AND CONTROLS.

1) AGE
2) SEX
3) BMI
4) DIABETES
5) HYPERTENSION
6) SMOKING
7) ALCOHOLISM
8) FASTING SUGARS
9) FASTING LIPID PROFILE
10) RENAL FUNCTION TESTS

STATISTICAL METHODS

The following statistical tests were used

1) t test
2) Chi-square test
3) Mean
4) Standard deviation
5) Pearson correlation to correlate between fibrinogen and other variables.

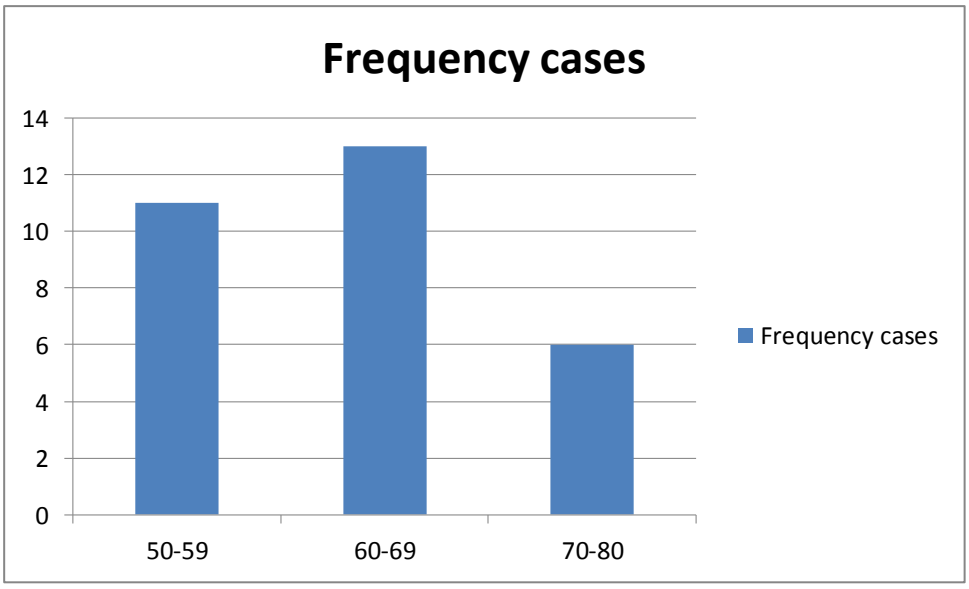
observations

AGE DISTRIBUTION AMONG CASES

TABLE 2: TABLE SHOWING AGE DISTRIBUTION OF CASES.

Age years	Frequency cases
50-59	11
60-69	13
70-80	6

FIGURE 2: BAR CHART SHOWING AGE DISTRIBUTION:



The mean age was 62.37(age range 50-76 years) in cases.

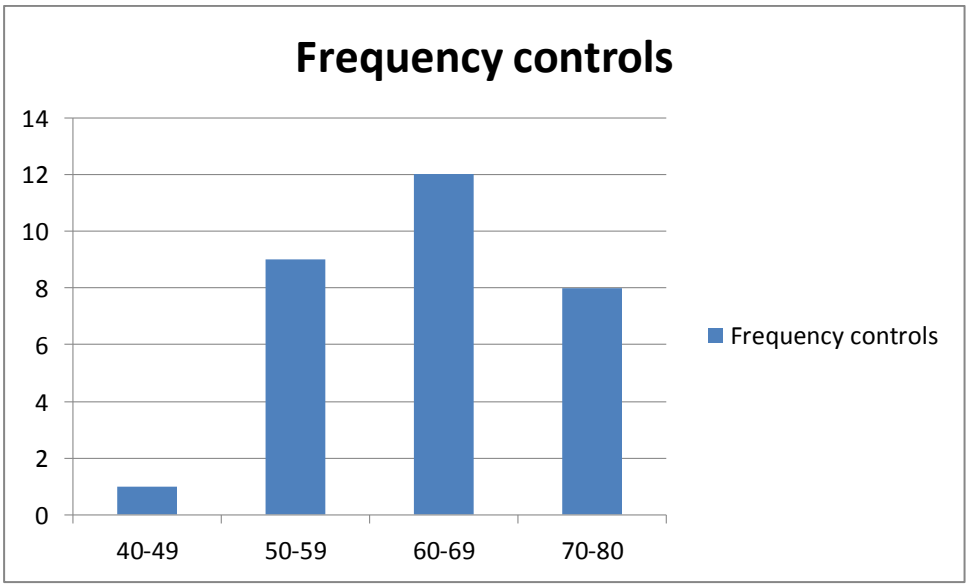
The maximum no of patients were in age group 60-69 years.

AGE DISTRIBUTION AMONG CONTROLS

TABLE 3: TABLE SHOWING AGE DISTRIBUTION OF CONTROLS.

Age in years	Frequency controls
40-49	1
50-59	9
60-69	12
70-80	8

FIGURE 3: BAR CHART SHOWING AGE DISTRIBUTION AMONG CONTROLS:



The mean age among controls was 62.87(age range 45-80 years).

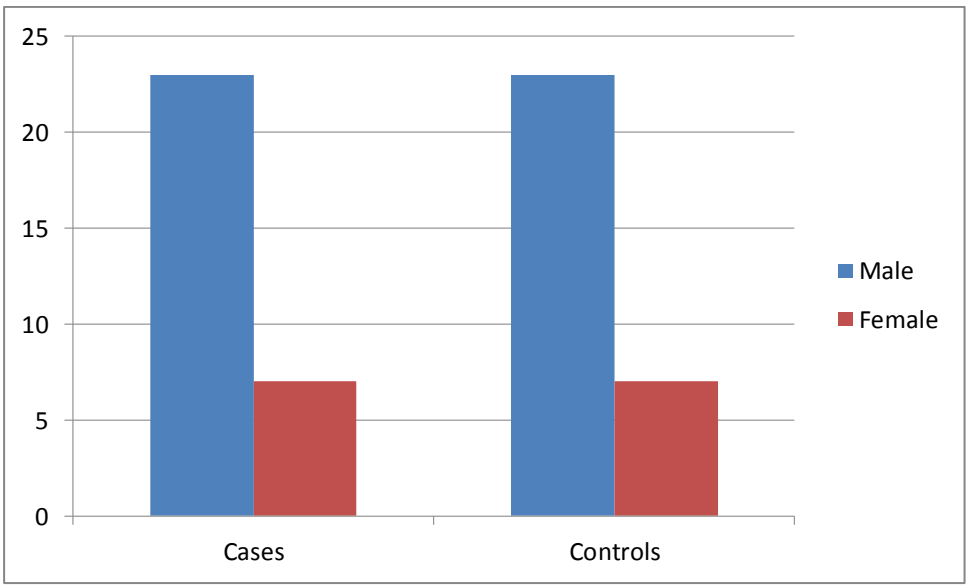
The maximum no of patients were in the age group 60-69 years.

SEX DISTRIBUTION AMONG CASES AND CONTROLS

TABLE 4: SHOWING SEX DISTRIBUTION AMONG CASES AND CONTROLS

Gender	Cases	Controls
Male	23	23
Female	7	7
Total	30	30

FIGURE 4: SHOWING SEX DISTRIBUTION AMONG CASES AND CONTROLS

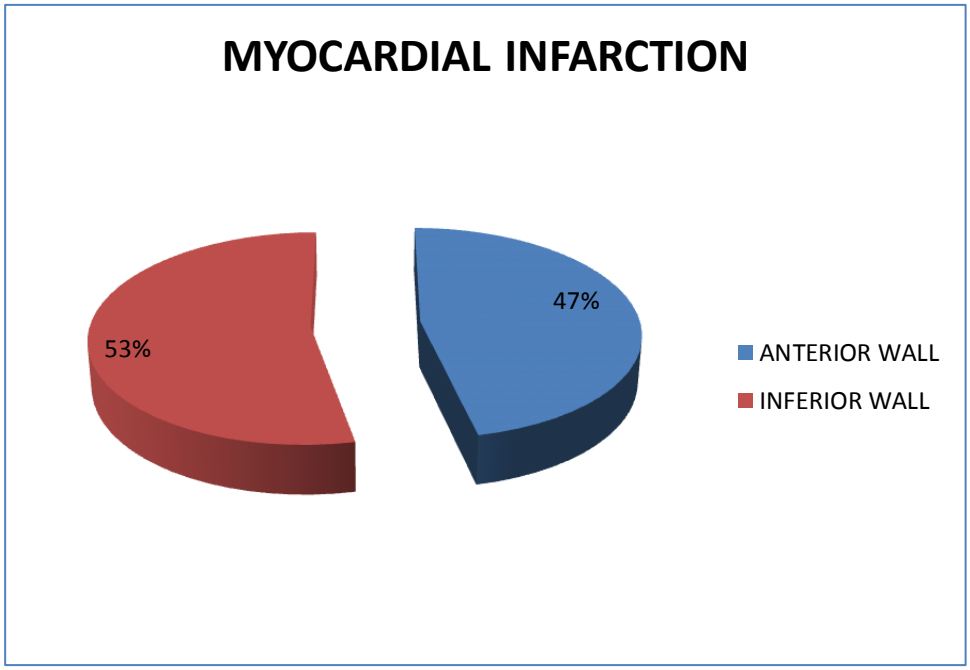


In this study among 30 cases studied, 23 were males and 7 were females, 23 were males and 7 were females in controls.

TABLE 5: SHOWING MYOCARDIAL INFARCTION AMONG CASES.

	CASES	
	N	**%**
	30	100.00%
ANTERIOR WALL	14	46.70%
INFERIOR WALL	16	53.30%

FIGURE 5: SHOWING MYOCARDIAL INFARCTION AMONG CASES AND CONTROLS.



Among the cases all had myocardial infarction (MI), 53% had anterior wall MI and 47% had inferior wall MI.

TABLE 6: SHOWING FIBRINOGEN LEVELS AMONG CASES AND CONTROLS.

	fibrinogen levels among cases	fibrinogen levels among controls
Minimum	241	183
Maximum	1415	543

In the present study, a minimum fibrinogen level among cases is 241mg/dl and 183mg/dl in controls.

Maximum fibrinogen level among cases is 1415mg/dl and 543mg/dl in controls.

TABLE 7: SHOWING MEAN FIBRINOGEN LEVELS IN CASES AND CONTROLS

Patients	Number	Mean	Standard deviation
Case	30	490.43	275.42
Control	30	292.2	104.789

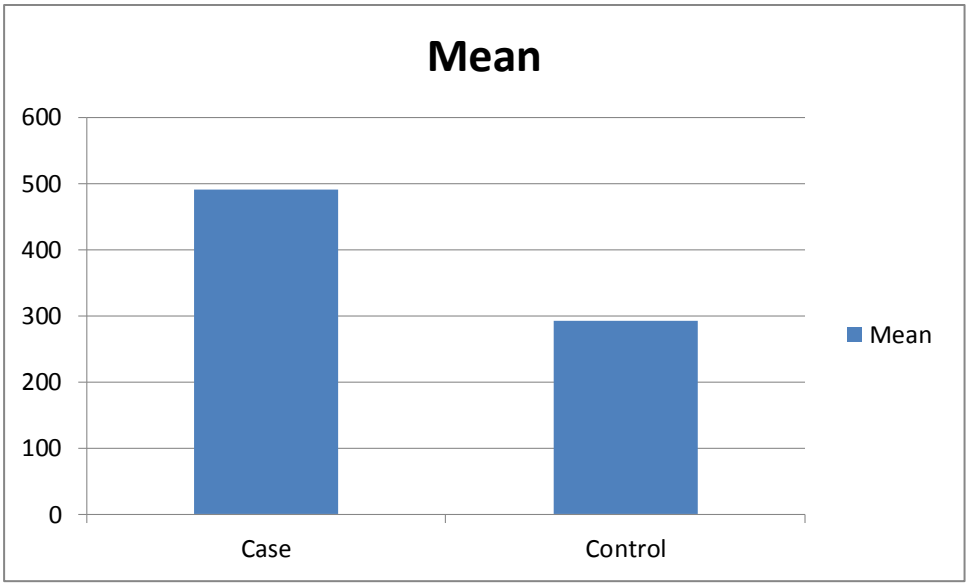
The mean plasma fibrinogen in cases was 490.43 ± 275.42.

The mean plasma fibrinogen in controls was 292.2 ± 104.789.

Cases had higher fibrinogen levels when compared to controls.

There was statistically significant difference between mean fibrinogen levels of cases and controls.

FIGURE 6: BAR CHART SHOWING MEAN FIBRINOGEN IN CASES AND CONTROLS.



AGE AND FIBRINOGEN LEVELS

TABLE 8: SHOWING CORRELATION BETWEEN AGE AND MEAN FIBRINOGEN LEVELS

Age group years	Frequency cases	Cases	Controls
50-59	11	602	259.5
60-69	13	450	306.5
70-80	6	373	311.5

TABLE 9: SHOWING CORRELATION BETWEEN FIBRINOGEN AND AGE

	Cases	**controls**
Pearson Correlation	**-0.215**	**0.107**
P value	**0.253**	**0.572**

In this study it was observed that in cases, fibrinogen levels decreases as age advances where as in controls, fibrinogen levels increases as age advances.

SEX AND FIBRINOGEN LEVELS

TABLE 10: SHOWING MEAN FIBRINOGEN(MG/DL) VALUES IN MALES AND FEMALES

	Cases	Controls
Males	509.4	281.9
Females	427.8 P=0.73	326 P=0.23

In cases males had mean fibrinogen of 509.4 and females had mean fibrinogen of 427. Males had higher fibrinogen levels when compared to females in cases, but not statistically significant.

In controls males had mean fibrinogen of 281.9 and females had mean fibrinogen of 326. Females had higher fibrinogen levels when compared to males in controls, but not statistically significant.

FIGURE 7: BAR CHARTS SHOWING MEAN FIBRINOGEN IN BOTH SEXES IN CASES.

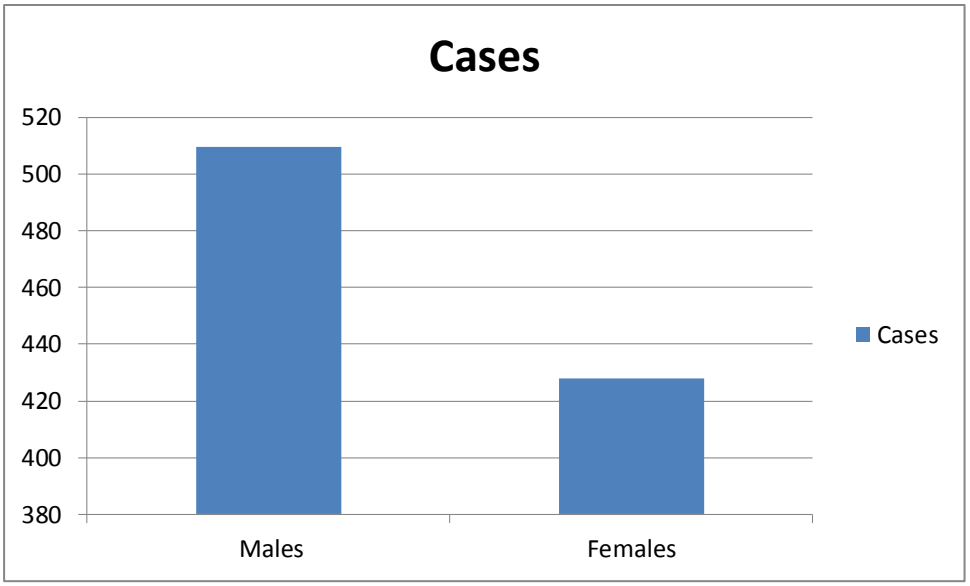
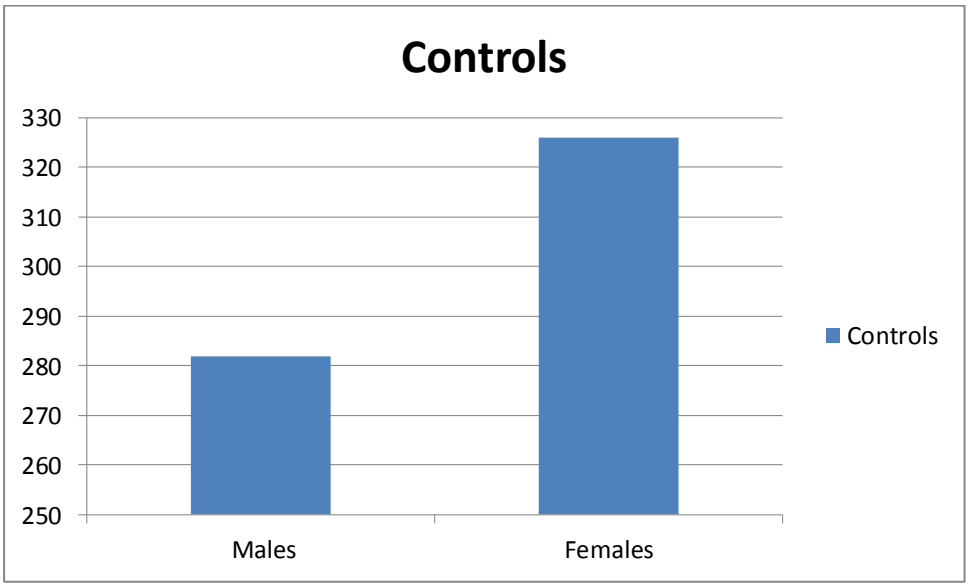


FIGURE 8: BAR CHART SHOWING MEAN FIBRINOGEN IN BOTH SEXES IN CONTROLS.



SMOKING WITH FIBRINOGEN

TABLE 11: SHOWING DISTRIBUTION OF SMOKERS IN CASES AND CONTROLS

	Case (N)	Case (%)	Control (N)	Control (%)	Chi square/p value
Non smokers	22	73.3%	21	70.0%	0.082
smokers	8	26.7%	9	30.0%	P=0.774

In this study 8(26.7%) were smokers in case group and 9(30%) were smokers in control group. Smoking was not significantly prevalent among cases when compared to controls.

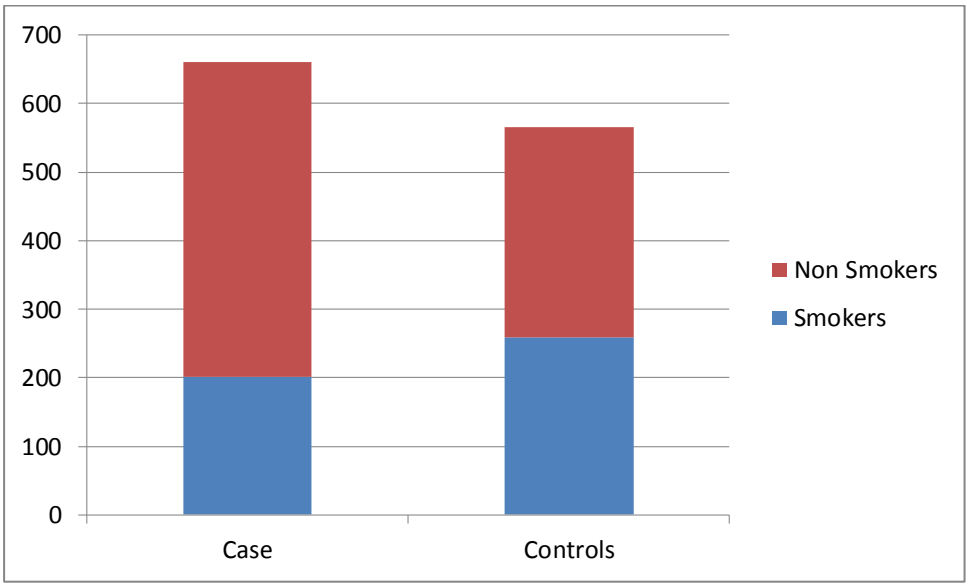
TABLE 12: SHOWING MEAN FIBRINOGEN (MG/DL) VALUE IN SMOKERS AND NON-SMOKERS

	Case	Controls
Smokers	202	259.5
Non Smokers	459 P=0.257	306.2 P=0.378

In cases smokers had mean fibrinogen of 202mg/dl, and nonsmokers had mean fibrinogen of 459mg/dl. Non-smokers had higher fibrinogen levels when compared to smokers in cases, but not statistically significant.

In controls smokers had mean fibrinogen of 259.5mg/dl and non-smokers had mean fibrinogen of 306.2. Non-smokers had higher fibrinogen levels when compared to smokers in controls, but not statistically significant.

FIGURE 9: SHOWING MEAN FIBRINOGEN IN SMOKERS AMONG CASES AND CONTROLS



HYPERTENSION

TABLE 13: SHOWING DISTRIBUTION OF HYPERTENSION AMONG CASES AND CONTROLS

	Case		Control		Chi square/p value
	N	%	N	%	
Non-Hypertensives	11	36.7%	21	70.0%	6.696*
Hypertensives	19	63.3%	9	30.0%	P=0.010

In this study, 19(63.3%) were hypertensives in case group and 11(36.7%) were hypertensives in control group. Hypertension was significantly prevalent among cases when compared to controls.

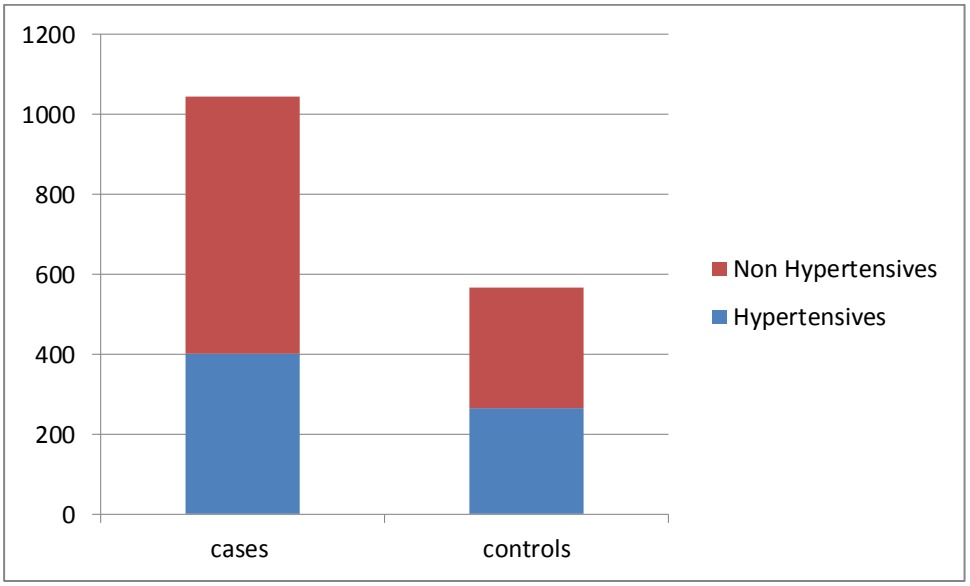
TABLE 14: SHOWING MEAN FIBRINOGEN LEVELS AMONG HYPERTENSIVES IN CASES AND CONTROLS.

	cases	controls
Hypertensive	401	265
Non Hypertensive	644 P=0.412	303 P=0.398

In cases hypertensives had mean fibrinogen of 401mg/dl, and non-hypertensives had mean fibrinogen of 644smg/dl. Non-hypertensives had higher fibrinogen levels when compared to hypertensives in cases, but not statistically significant.

In controls hypertensives had mean fibrinogen of 265mg/dl and non-hypertensives had mean fibrinogen of 303mg/dl. Non-hypertensives had higher fibrinogen levels when compared to hypertensives s in controls, but not statistically significant.

FIGURE 10: SHOWING MEAN FIBRINOGEN LEVELS AMONG CASES AND CONTROLS IN HYPERTENSIVES AND NON HYPERTENSIVES



DIABETES

TABLE 15: SHOWING DISTRIBUTION OF DIABETES AND NON DIABETICS AMONG CASES AND CONTROLS

	Case (N)	Case %	Control(N)	Control (%)	Chi square/p value
Non-Diabetics	13	43.3%	20	66.7%	3.30
Diabetics	17	56.7%	10	33.3%	P=0.069

In this study, 17(56.7%) were diabetics in case group and 10(33.3s%) were diabetics in control group.Diabetes was not significantly prevalent among cases when compared to controls.

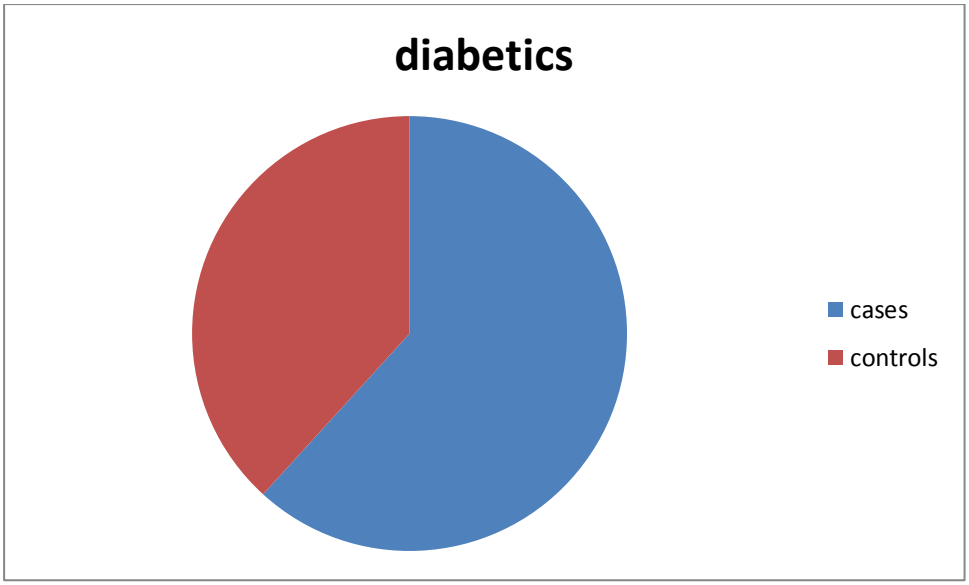
TABLE 16: SHOWING MEAN FIBRINOGEN LEVELS AMONG DIABETES AND NON-DIABETICS IN CASES AND CONTROLS.

	cases	controls
diabetics	512	317
non diabetics	461 P=0.356	279 P=0.459

In cases diabetics had mean fibrinogen of 512mg/dl, and non-diabetics had mean fibrinogen of 461mg/dl. Diabetics had higher fibrinogen levels when compared to non-diabetics in cases, but not statistically significant.

In controls diabetics had mean fibrinogen of 317mg/dl and non-diabetics had mean fibrinogen of 279mg/dl. Diabetics had higher fibrinogen levels when compared to non-diabetics s in controls, but not statistically significant.

FIGURE 11: SHOWING MEAN FIBRINOGEN LEVELS AMONG DIABETES AND NON-DIABETICS IN CASES AND CONTROLS.



BODY MASS INDEX

TABLE 17: SHOWING DISTRIBUTION OF OBESITY AMONG CASES AND CONTROLS

Group	N	Mean	Std Deviation		t value	P value
Case	30	28.05	2.48		4.308*	P<0.001
Control	30	25.27	2.52			

In this study, obesity was significantly prevalent among cases when compared to controls.

TABLE 18: SHOWING MEAN FIBRINOGEN LEVELS AMONG OBESE AND NON-OBESE IN CASES AND CONTROLS.

	Cases(N)	Cases (mean fibrinogen)	Controls(N)	Controls (mean fibrinogen)
non obese	2	522	16	266
obese	28	488 P= 0.345	14	322 P=0.297

In cases obese (N=28) group had mean fibrinogen of 488mg/dl, and non- obese (N=2) had mean fibrinogen of 522mg/dl. Non-Obese group had higher fibrinogen levels when compared to obese in cases, but not statistically significant. Although majority of the patients in cases belonged to obese group.

In controls obese (N=14) had mean fibrinogen of 322mg/dl and non- obese (N=16) had mean fibrinogen of 266mg/dl. Obese had higher fibrinogen levels when compared to non-obese in controls, but not statistically significant.

TABLE 19: SHOWING CORRELATION BETWEEN BMI AND FIBRINOGEN LEVELS.

		Cases	**Controls**
BMI(kg/m^2)	Pearson Correlation	**-0.117**	**-0.052**
	P value	**0.538**	**0.785**

Both in cases and controls, fibrinogen decreased with increasing BMI.

FIGURE 12: PIE CHART SHOWING MEAN FIBRINOGEN AMONG OBESE AND NON-OBESE AMONG CASES

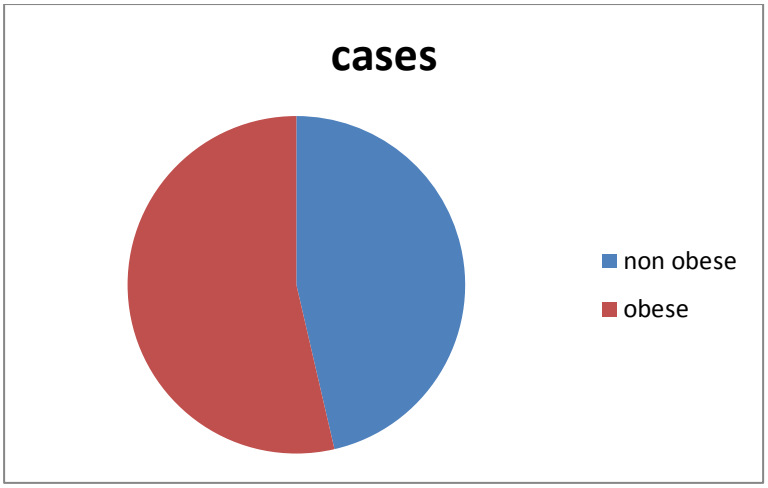
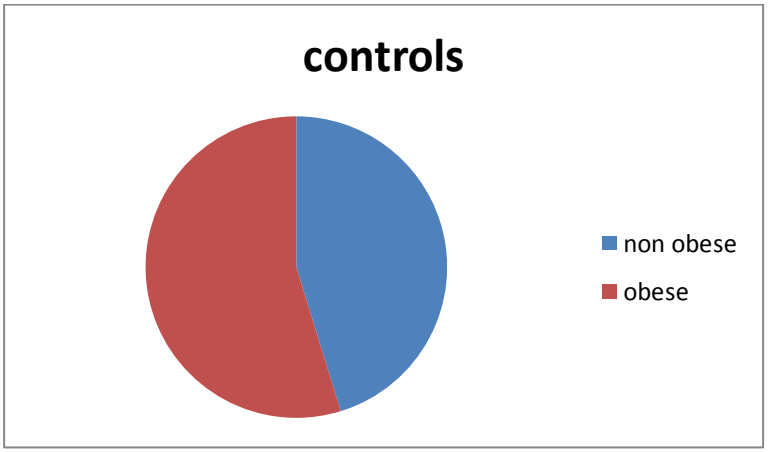


FIGURE 13: PIE CHART SHOWING MEAN FIBRINOGEN IN OBESE AND NON-OBESE AMONG CONTROLS.



FIBRINOGEN WITH OTHER PARAMETERS

Fasting sugars were also more in cases (mean=162.03) compared to controls (mean=97.90).

Further, cholesterol, LDL, triglycerides and were higher in cases when compared to controls.

Fasting sugar was positively correlating with fibrinogen in both cases (Pearson Correlation=0.046, P value= 0.811) and controls (Pearson Correlation = 0.120, P value=0.528) though not statistically significant.

Among cases, cholesterol (Pearson Correlation= -0.010, P value=0.957), HDL(Pearson Correlation= -0.142, P value=0.454) were negatively correlating, triglycerides(Pearson Correlation=0.097,P=0.611) and LDL(Pearson Correlation=0.076, P value=0.689) were positively correlating with fibrinogen levels though not statistically significant.

Among controls, cholesterol (Pearson Correlation= -0.298, P value=0.110), HDL (Pearson Correlation= -0.023, P value=0.904) and LDL (Pearson Correlation=-0.330, P value=0.075) were negatively correlating, triglycerides (Pearson Correlation=0.080, P=0.673) was positively correlating with fibrinogen levels though not statistically significant.

DISCUSSION

In this study, 30 cases and 30 controls were included. The mean fibrinogen levels in cases was 490.43mg/dl and 292.2mg/dl among controls. The difference was statistically significant.

A major meta-analysis involving 31 prospective study [51], moderately strong associations were found between usual plasma fibrinogen level and the risks of CHD, stroke, other vascular mortality, and nonvascular mortality in a wide range of circumstances in healthy middle-aged adults. Another major meta-analysis[52] involving 6 prospective studies concluded that all prospective studies showed plasma fibrinogen was associated with subsequent myocardial infarction (MI) or stroke. The North wick park heart study[53], Leigh general practice study [56], Framingham study [57,58], all proved fibrinogen to be independently associated with adverse cardiovascular events.

TABLE 20: SHOWING PLASMA FIBRINOGEN LEVEL IN OTHER STUDIES IN COMPARISION TO PRESENT STUDY.

Study	Study population	Mean fibrinogen cases(mg%)	Mean fibrinogen controls(mg%)	P value
NPHS study	1511	315	290	<0.001
Gothenberg	792	356	330	<0.001
Leigh	297	392	313	<0.001
Speedwell	226 223	287 439	297 402	NS <0.01
Present study	60	490	292	<0.001

In all the above studies the level of fibrinogen in cases is increased when compared to controls which was statistically significant. In Leigh and speed well studies nephelometry method was used to assess fibrinogen among study population. In our study mean fibrinogen levels were higher compared to those studies.

Causes for variation could be

1) Our study was conducted in eastern, south Indian population, above said studies were conducted in western population though the method of estimation was same in leigh, and speed well studies.

2) Large study population in above studies.

Age and fibrinogen

Plasma concentrations of fibrinogen generally increase with age [69, 71, 74, 75]. This age-related increase in plasma fibrinogen may be due to a slower rate of disposal of fibrinogen, rather than an increased production rate [64].

This study demonstrated that fibrinogen levels decreased as age advances in cases but in cases fibrinogen levels increased as age advances.

Gender and fibrinogen

The second World Health Organization Monitoring Trends and Determinants in Cardiovascular Disease (MONICA) Augsburg survey found the crude fibrinogen values to be consistently higher in women than in men of all ages, irrespective of pregnancy or the use of oral contraceptives.[69-73] . Furthermore, this pattern was observed even among healthy adolescents in the Florence Teenager Study.[70] .However, occasional studies have failed to demonstrate a significant gender difference in plasma fibrinogen levels between men and women.[71]

In our study males had higher fibrinogen levels when compared to females in cases and females had higher fibrinogen levels when compared to males in controls.

Smoking

Available evidence suggests that cigarette smoking is strongly associated with increased plasma fibrinogen levels, and the adverse cardiovascular effects of smoking may partly be mediated through an increase in plasma fibrinogen levels. [79-81].Indeed, each cigarette smoked per day increases mean plasma fibrinogen by 0.35 g/l. [71]. Similar data are available from epidemiological studies [82-94] showed that plasma fibrinogen values were significantly higher in smokers than in non-smokers, with a dose-dependent increase with smoking in both sexes; ex-smokers had values as low as

those of non-smokers. Furthermore, smoking could have an acute effect on plasma fibrinogen levels. For example, post-MI patients who smoked within the previous 24 h had significantly higher plasma fibrinogen levels than patients who refrained from smoking for 24 h. [87]

In this study, non-smokers had higher fibrinogen levels when compared to smokers in both cases, and controls though not statistically significant.

EFFECT OF SMOKING ON PLASMA FIBRINOGEN LEVELS

1) Smoking increases the production of the cytokines, such as interleukin-6 [90]. which plays a major role in increasing synthesis of acute phase protiens from Liver including fibrinogen [91,92].

2) Smoking results in an inflammatory reaction, resulting in an increase in C-reactive protien [94].

3) Endothelial dysfunction [94]

4) Altering the activity of platelets.[94]

5) Increasing plasma fibrinogen concentration[94].

Hypertension and fibrinogen

A meta-analysis[51], an Australian study[68], showed that fibrinogen levels correlated with several established risk factors like Hypertension.

In our study, Hypertension was significantly prevalent among cases when compared to controls. Non-hypertensives had higher fibrinogen levels when compared to hypertensives in both cases and controls, but not statistically significant.

The mechanisms associated with elevated fibrinogen levels and hypertension could be

1) Relation of fibrinogen to increased viscosity and peripheral vascular resistance

2) Decreased fibrinolytic activity in hypertensives due to hyperinsulinemia in hypertensives.

3) IL-6 and IL-8 are elevated in hypertension and causes reduced degradation of fibrinogen. Hence increased fibrinogen levels in hypertensives.

Diabetes and fibrinogen

The Framingham study [57,58], proved that impact of plasma fibrinogen levels on cardiovascular disease was comparable with the major risk factors, such as blood pressure, haematocrit, adiposity, cigarette smoking and **diabetes**. In a meta-analysis[51], plasma fibrinogen was associated with 'true' risk factors such as diabetes, hypertension and hypercholesterolemia in the studies included in this meta-analysis.

In this study, diabetes was not significantly prevalent among cases when compared to controls. Diabetics had higher fibrinogen levels when compared to non-diabetics in cases, but not statistically significant.

The exact mechanism of increased fibrinogen levels in diabetics is unknown,

possible mechanisms includes:,

1. Insulin stimulates cholesterol synthesis in smooth muscle cells and macrophages of the arterial walls, stimulates the proliferation and migration of smooth muscle cells. It also enhances the formation of fibrinogen.

2. Endothelial dysfunction which is common in diabetics, which causes decreased fibrinolytic activity and hence increased plasma fibrinogen levels.

3. The plasma glucagon concentration is positively related to the plasma fibrinogen concentration. Thus, fibrinogen production is markedly enhanced in diabetic patients, and this alteration is likely to determine the observed hyperfibrinogenemia in these patients. Hyperglucagonemia may contribute to the increased fibrinogen production.

Obesity and fibrinogen

Plasma fibrinogen concentration has been positively correlated with body mass index, the waist circumference, the hip circumference and waist-to-hip ratio in both sexes.[69,75,77]. Indeed, plasma fibrinogen level is significantly higher amongst patients

with a body mass index of > 30 kg/m2 , compared to those with body mass index < 25 kg/m2[78].

In the present study, BMI was significantly more prevalent among cases when compared to controls. In cases, non-Obese group had higher fibrinogen levels when compared to obese, but not statistically significant. Although majority of the patients in cases belonged to obese group.

In controls, obese had higher fibrinogen levels when compared to non-obese in controls, but not statistically significant.

CONCLUSION

1) In this study mean plasma fibrinogen levels were significantly higher in coronary artery disease patients when compared to controls.
2) Other conventional risk factors for CAD are not having higher levels of fibrinogen.

SUMMARY

1) Coronary artery disease incidence increases as the age advances and is more common in males than females.

2) Plasma fibrinogen levels are elevated in coronary artery disease patients.

BIBLIOGRAPHY

1) Yusuf S, Hawken S, Ounpuu S. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): Case-control study. Lancet 2004; 364:937.

2) Bhatt DL, Steg PG, Ohman EM, Rather J, Wilson PW. International prevalence, recognition, and treatment of cardiovascular risk factors in outpatients with atherothrombosis. JAMA 2006; 295:180.

3) Ridker P M , Libby P. Risk factors for atherothrombotic disease. Zipes D P, Ubby P, Bonow, Braunwald E. Braunwald's heart disease, t W, Philadelphia: Elsevier saunders , 2007:1004.

4) Margagilone M, Cappuci G, Collaizo D. Fibrinogen plasma levels in an apparently healthy general population: Relation to environmental and genetic determinants. Thromb Haemost 80:805, 1998.

5) Folsom AR, Wu kk, Rosamond WD, Sharrett AR, Chamless LE. Prospective study of hemostatic factors and incidence of IHD. The Atherosclerosis risk in communities (ARIC) study. Circulation.1997; 96:1102-1108.

6) Mossesan MW. Fibrinogen and fibrin polymerization: appraisal of the binding events that accompany fibrin generation and fibrin clot assembly. Blood coagul Fibrinolysis.1997 Jul;8(5): 256-67.

7) Henschen A, Lottspeich F. Kehl M, Southan C. Covalent structure of fibrinogen. Ann N Y Acad Sci 1983; 408: 28—43.

8) Blomback B, Hessel B, Hogg D. Disulfide bridges in NH2-terminal part of human fibrinogen. Thromb Res 1976; 8: 639—58.

9) Huang S, Cao Z, Davie EW. The role of amino-terminal disulfide bonds in the structure and assembly of human fibrinogen. Biochem Biophys Res Commun. 1993; 190: 488—95.

10) Zhang J-Z. Redman CM. Identification of BŠ chain domains involved in human fibrinogen assembly. J Biol Chem. 1992; 267: 21727—32.

11) Hoeprich PD Jr, Doolittle RF. Dimeric half-molecules of human fibrinogen are joined through disulflde bonds in an antiparallel orientation. Biochemistry 1983; 22: 2M9—55.

12) Scheraga HA, Laskowski NI Jr. The fibrinogen-fibrin conversion. Adv protein chem. 1957; 12: 1—131.

13) Blomback B. Studies on the action of thrombotic enzymes on bovine fibrinogen as measured by N-terminal analysis. Ark Kemi. 1958; 12: 321-35.

14) Blornbäck B, Hessel B, Hogg D. Therkildsen L. A two-step fibrinogen fibrin transition in blood coagulation. Nature. 1978: 275: 501-5.

15) Shimizu A, Nagel GM, Doolittle RF. Photoaffinity labeling of the primary fibrin polymerization site: isolation of a CNBr fragment corresponding to c337–379. Proc Natl Acad Sci U S A 1992; 89: 2888–92.

16) Pratt KR, Cote HCF, Chung DW, Stenkamp RE, Davie EW. The primary fibrin polymerization pocket: three-dimensional structure of a 30-kDa C-terminal y-chain fragment complexed with the peptide gly-pro-arg-pro. Proc Natl Acad Sci U S A. 1997; 94: 7176—81.

17) Everse SJ. Spraggon G, Veerapandian L, Riley M, Doolittle RF. Crystal structure of fragment double-D from human fibrin with two different bound ligands. Biochemistry. 1998; 37: 8637-42.

18) Ferry JD. The mechanism of polymerization of fibrinogen. Proc Natl Acad Sci U S A 1952; 38: 566–9.

19) Krakow W, Endres GF, Siegel BM, Scheraga HA. An electron microscopic investigation of the polymerization of bovine fibrin monomer. J Mol Biol. 1972; 71: 95–103.

20) Fowler WE, Hantgan RR, Hermans J, Erikson HP. Structure of the fibrin protofibril. Proc Natl Acad Sci U S A 1981; 78: 4872–6.

21) Muller MF, Ris HA, Ferry JD. Electron microscopy of fine fibrin clots and fine and coarse fibrin films. J Mol Biol 1984; 174: 369–84.

22) Mosesson MW, Siebenlist KR, Amrani DL, DiOrio JP. Identification of covalently linked trimeric and tetrameric D domains in cross linked fibrin. Proc Natl Acad Sci U S A 1989; 86: 1113–7.

23) Hewat EA, Tranqui L, Wade RH. Electron microscope structural study of modified fibrin and a related modified fibrinogen aggregate. J Mol Biol 1983; 170: 203–22.

24) Mosesson MW, DiOrio JP, Siebenlist KR, Wall JS, Hainfeld JF. Evidence for a second type of fibril branch point in fibrin polymer networks, the trimolecular junction. Blood 1993; 82: 1517–21.

25) Mosesson MW, DiOrio JP, Muller MF, Shainoff JR, Siebenlist KR, Amrani DL, Homandberg GA, Soria J, Soria C, SamamaM. Studies on the ultrastructure of fibrin lacking fibrinopeptide B (b-fibrin). Blood 1987; 69: 1073–81.

26) de Maat MP, Kastelein JJ, Jukema JW, Zwinderman AH, Jansen H, Groenemeier B, et al. 455G/A polymorphisms of the B-fibrinogen gene is associated with the progression of coronary atherosclerosis in symptomatic men. Arterioscler, Thromb, and Vasc Biol. 1998;18: 265-271.

27) 11. Brown ET, Fuller GM. Detection of a complex that associates with the Bß fibrinogen G-455-A polymorphism. Blood. 1998;92:3286–3293.

28) 12. van 't Hooft FM, von Bahr SJ, Silveira A, Iliadou A, Eriksson P, Hamsten A. Two common, functional polymorphisms in the promoter region of the ß-fibrinogen gene contribute to regulation of plasma fibrinogen concentration. Arterioscler Thromb Vasc Biol. 1999;19: 3063–3070.

29) 13. Behague I, Poirier O, Nicaud V, Evans A, Arveiler D, Luc G et al.ß-Fibrinogen gene polymorphisms are associated with plasma fibrinogen and coronary artery disease in patients with myocardial infarction: The ECTIM Study. Etude Cas-Temoins sur l'Infarctus du Myocarde. Circulation. 1996;93: 440–449.

30) 14. Endler G, Mannhalter C. Polymorphisms in coagulation factor genes and their impact on arterial and venous thrombosis. Clin Chim Acta. 2003;330:31–55.

31) 15. Boekholdt SM, Bijsterveld NR, Moons AH, Levi M, Buller HR, Peters RJ. Genetic variation in coagulation and fibrinolytic proteins and their relation with acute myocardial infarction: a systematic review. Circulation. 2001;104:3063–3068.

32) 17. Martiskainen M, Pohjasvaara T, Mikkelsson J, Mantyla R, Kunnas T, Laippala P. Fibrinogen gene promoter -455 A allele as a risk factor for lacunar stroke. Stroke. 2003;34:886–891.

33) Jacquemin B, Antoniades C, Nyberg F, Plana E, Muller M, Greven S et al. Common Genetic Polymorphisms and Haplotypes of Fibrinogen Alpha, Beta, and Gamma Chains Affect Fibrinogen Levels and the Response to Proinflammatory Stimulation in Myocardial Infarction Survivors.The AIRGENE Study. J Am Coll Cardiol 2008;52:941–52.

34) Clauss Va. Gerinnungsphysiologishe schnellmethode zur Bestimmung des Fibrinogens. Acta Haematol. 1957; 17 : 237-246.

35) Gaffney PJ, Wong MY. Collaborative study of a proposed international standard fpr plasma fibrinogen measurement. Thromb Haemost. Thromb Haemost.1992;68:428-432.

36) Jacobsson K. studies in the determination of fibrinogen in human blood plasma. Scand J Clin Lab Invest Suppl. 1955; 7: 1-54.

37) Blomback B, Blomback M. PurifiCation of human and bovine fibrinogen. Arkiv Kemi. 195; 10: 415-443.

38) Rossi E, Mondonico P,Lombardi A, et al. Method for the determination of functional(clottable) fibrinogen by the new family of ACL coagulometers. Thromb Res. 1988;52: 453-468.

39) Parareti G, Maccaferri M, et al. Fibrinogen assays a collaborative study of six different study of six different methods. Clin chem. 1991; 37: 714-719.

40) Bovill EG, Mc Donagh J, Triplett DA,et al. performance characteristics of fibrinogen assays: Results of college of American Pathologists proficiency program. 1988-1991. Arch Pathol Lab Med. 1993; 117: 58-66.

41) Wu KK, Folsom AR, HeissG, Davis CE, for the ARIC study investigators. Association of coagulation factors and inhibitors with carotid Artery Atherosclerosis Risk in Communities(ARIC) study. Ann Epidemiol.1992;2: 471-480.

42) Lowe G D, Drummond MN, Bremer WF. Increased plasma fibrinogen and platelet aggregates in type II hyperlipoproteinemia. Thromb Haemostas. 1979; 42: 1503-1507.

43) Ernst E, Matral A, Scholzi C. Dose effect relationship between smoking and blood rheology. Br J Haematol.1987; 65: 485-7.

44) Ernst E, Matral A. Abstinence from chronic cigarette smoking normalizes blood rheology, Atherosclerosis. 1988; 8: 385-8.

45) Woodward M, Lowe GDO, Rumley A, Epidemiology of coagulation factors, inhibitors and activation markers: The Third Glasgow MONICA survey II. Relationships to cardiovascular disease. Br J Haematol.1997; 97: 785-797.

46) Kannel WB, D' Agostino RB, Belanger AJ, Fibrinogen and risk of cardiovascular disease. The Framingham Study. JAMA. 1987;258: 1183-1186.

47) Kannel WB, D'Agostio RB, Belanger AJ. Fibrinogen, cigarette smoking and risk of cardiovascular disease: insights from Framingham study. AM Heart J. 1987; 113: 1006-10.

48) Landin K, Tengborn L, Smith U. Elevated fibrinogen and plasminogen activator inhibitor (PAI-I) in hypertension are related to metabolic risk factors for cardiovascular disease. J Intern Med. 1990; 120: 672-6.

49) Kannel WB, D'Agostio RB, Wilson P W, Belanger AJ, Gagnon D R. Diabetes, Fibrinogen and risk for cardiovascular diasease: The Framigham experience. Am Heart J. 1990; 120: 672-6.

50) Schmitz A, Ingerslev J, Hemostatic measurements in type II diabetic patients with microalbuminuria. Diabet Med. 1990; 7: 521-5.

51) Danesh J, Lewington S, Thompson S G, Lowe G D O, Collins R. Plasma Fibrinogen Level and the Risk of Major Cardiovascular Diseases and Nonvascular Mortality. An Individual Participant Meta-analysis. JAMA. 2005;294:1799-1809.

52) Ernst E, Resch KL Fibrinogen as a cardiovascular risk factor: a meta-analysis and review of the literature. Ann Intern Med. 1993; 118:956–63.

53) Meade TW, Mellows S, Brozovic M, Miller GJ, Chakrabarti RR, North WR et al. Haemostatic function and ischaemic heart disease: principal results of the Northwick Park Heart Study. Lancet. 1986; 2:533–7.

54) Lee AJ, Lowe GD, Woodward M, Tunstall-Pedoe H. Fibrinogen in relation to personal history of prevalent hypertension, diabetes, stroke, intermittent claudication, coronary heart disease, and family history: the Scottish Heart Health Study. Br Heart J. 1993; 69:338–42.

55) Wilhelmsen L, Svardsudd K, Korsan-Bengtsen K, Larsson B, Welin L, Tibblin G. Fibrinogen as a risk factor for stroke and MI. The Gothenburg study. N Engl J Med 1984; 311:501–5.

56) 41.Stone MC, Thorp JM. Plasm fibrinogen-a major coronary risk factor. J Roy Coll Gen Prac. 1985; 35:565–9.

57) 42. Kannel WB, Wolf PA, Castelli WP, D'Agostino RB. Fibrinogen and risk of cardiovascular disease. The Framingham Study. JAMA 1987; 258:1183–6.

58) 43Kannel WB, D'Agostino RB, Wilson PW, Belanger AJ, Gagnon DR. Diabetes, fibrinogen, and risk of cardiovascular disease: the Framingham experience. Am Heart J 1990;120:672–6.

59) 44 Assmann G, Cullen P, Heinrich J, Schulte H. Hemostatic variables in the prediction of coronary risk: results of the 8 year follow-up of healthy men in the Munster Heart Study(PROCAM). Prospective Cardiovascular Munster Study. Isr J Med Sci 1996; 32:364–70.

60) 45 Yarnell JW, Baker IA, Sweetnam PM, Bainton D, O'Brien JR, Whitehead PJ, et al. Fibrinogen, viscosity, and white blood cell count are major risk factors for ischemic heart disease. The Caerphilly and Speedwell collaborative heart disease studies. Circulation. 1991; 83:836–44.

61) Thompson SG, Kienast J, Pyke SD, Haverkate F, van de Loo Hemostatic factors and the risk of MI or sudden death in patients with angina pectoris. European Concerted Action on Thrombosis and Disabilities Angina Pectoris Study Group.N Engl J Med. 1995; 332:635–41.

62) Russell P. Tracy, Alice M. Arnold, Walter Ettinger, Fried L. The Relationship of Fibrinogen and Factors VII and VIII to Incident Cardiovascular Disease and Death in the Elderly Results From the Cardiovascular Health Study. Arterioscler Thromb Vasc Biol. 1999;19:1776-1783.

63) Lee AJ, Fowkes FG, Lowe GD, Connor JM, Rumley A.Fibrinogen, factor VII and PAI-1 genotypes and the risk of coronary and peripheral atherosclerosis: Edinburgh Artery Study. Thromb Haemost. 1999; 81:553-560.

64) Shinichi Sato S , Nakamura M, Lida M, Naito Y. Plasma Fibrinogen and Coronary Heart Disease in Urban Japanese. Am J Epidemiol 2000;152:420-3.

65) Kaur S, Singh P, Indu V, Singla G.Fibrinogen, Lp(a), Microalbuminuria and Left Ventricular Mass Index: Cardiovascular Disease Risk factors in Diabetes. Ind J Clin Biochem (Jan-Mar 2012) 27(1):94–96.

66) Green D, Chan C, Kang J, Liu K, Schreiner P, Jenny N S. Longitudinal assessment of fibrinogen in relation to subclinical cardiovascular disease: the CARDIA study. J Thromb Haemost. 2010 March ; 8(3): 489–495.

67) Andre P Kengne A P, Czernichow S, Stamatakis E, Hamer M, Batty G D. Fibrinogen and future cardiovascular disease in people with diabetes: Aetiological associations and risk prediction using individual participant data from nine community-based prospective cohort studies. Diabetes & Vascular Disease Research.2012; 0(0):1-9.

68) Brown L J M,Cunningham J, Nandi N, Hodge A.Fibrinogen and associated risk factors in a high-risk population: urban indigenous Australians, the druid Study. Cardiovascular Diabetology. 2010; 9:69-74.

69) Krobot K, Hense HW, Cremer P, Eberle E, Keil U. Determinants of plasma fibrinogen: relation to body weight,waist-to-hip ratio, smoking, alcohol, age, and sex. Results from the second MONICA Augsburg survey 1989–1990. Arterioscler Thromb 1992; 12:780–8.

70) Prisco D, Fedi S, Brunelli T, et al. Fibrinogen and factor VIIag in healthy adolescents: the Floren-teen (Florence teenager) Study. Thromb Haemost 1996; 75:778–81.

71) Tarallo P, Henny J, Gueguen R, Siest G. Reference limits of plasma fibrinogen. Eur J Clin Chem Clin Biochem 1992;30:745–51.

72) Laharrague PF, Cambus JP, Fillola G, Corberand JX Plasma fibrinogen and physiological aging. Aging (Milano) 1993;5:445–9.

73) Giansante C, Fiotti N, Cattin L, Da Col PG, Calabrese S.Fibrinogen, D-dimer and thrombin-antithrombin complexes in a random population sample: relationships with other cardiovascular risk factors. Thromb Haemost 1994;71:581–6.

74) Ishikawa S, Kario K, Nago N, et al. Factor VII and fibrinogen levels examined by age, sex, and other atherosclerotic risk factors in a Japanese population. The Jichi Medical School Cohort Study. Thromb Haemost 1997; 77:890–3.

75) Balleisen L, Bailey J, Epping PH, Schulte H, van de Loo J. Epidemiological study on factor VII, factor VIII and fibrinogen in an industrial population: I. Baseline data on the relation to age, gender, body-weight, smoking, alcohol, pill-using, and menopause. Thromb Haemost 1985; 54:475–9.

76) Fu A, Sreekumaran Nair K. Age effect on fibrinogen and albumin synthesis in humans. Am J Physiol 1998; 275(6 Pt 1):E1023–30.

77) Ditschuneit HH, Flechtner-Mors M, Adler G. Fibrinogen in obesity before and after weight reduction. Obes Res 1995;3:43–8.

78) Craveri A, Tornaghi G, Paganardi L, Ranieri R, Leonardi G, Di Bella M. Hemorrheologic disorders in obese patients. Study of the viscosity of the blood, erythrocytes, plasma fibrinogen and the erythrocyte filtration index. Minerva Med.1987; 78:899–906.

Eliasson M, Asplund K, Evrin PE, Lundblad D. Relationship of cigarette smoking and snuff dipping to plasma fibrinogen, fibrinolytic variables and serum insulin. The Northern Sweden MONICA Study. Atherosclerosis 1995; 113:41–53.

79) Cigolini M, Targher G, de Sandre G, Muggeo M, Seidell JC. Plasma fibrinogen in relation to serum insulin, smoking habits and adipose tissue fatty acids in healthy men. Eur J Clin Invest 1994; 24:126–30.

80) Dotevall A, Kutti J, Teger-Nilsson AC, Wadenvik H, Wilhelmsen L. Platelet reactivity, fibrinogen and smoking. Eur J Haematol 1987; 38:55–9.

81) Kannel WB, D'Agostino RB, Belanger AJ. Fibrinogen,cigarette smoking, and risk of cardiovascular disease:insights from the Framingham Study. Am Heart J 1987;113:1006–10.

82) Cullen P, Schulte H, Assmann G. Smoking, lipoproteins and coronary heart disease risk. Data from the Munster Heart Study (PROCAM). Eur Heart J 1998; 19:1632–41.

83) Meade TW, Imeson J, Stirling Y. Effects of changes in smoking and other characteristics on clotting factors and the risk of ischaemic heart disease. Lancet 1987; 2:986–8.

84) Kaufman DW, Palmer JR, Rosenberg L, Shapiro S. Cigar and pipe smoking and MI in young men. Br Med J (Clin Res Ed). 1987; 294:1315–16.

85) Iso H, Shimamoto T, Sato S, Koike K, Iida M, Komachi Y. Passive smoking and plasma fibrinogen concentrations. Am J Epidemiol 1996; 144:1151–4.

86) Fisher SD, Zareba W, Moss AJ, Marder VJ, Sparks CE, Hochman J,et al. Effect of smoking on lipid and thrombogenic factors two months after acute MI. Am J Cardiol 2000; 86:813–18.

87) Das I. Raised C-reactive protein levels in serum from smokers. Clin Chim Acta 1985; 153:9–13.

88) Mendall MA, Patel P, Ballam L, Strachan D, Northfield TC. C reactive protein and its relation to cardiovascular risk factors: a population based cross sectional study. Br Med J 1996; 312:1061–5.

89) McCarty MF. Interleukin-6 as a central mediator of cardiovascular risk associated with chronic inflammation, smoking, diabetes, and visceral obesity: down-regulation with essential fatty acids, ethanol and pentoxifylline. Med Hypotheses 1999; 52:465–77.

90) Castell JV, Gomez-Lechon MJ, David M, Andus T, Geiger T, Trullenque R,et al. Interleukin-6 is the major regulator of acute phase protein synthesis in adult human hepatocytes. FEBS Lett 1989; 242:237–9.

91) Marinkovic S, Jahreis GP, Wong GG, Baumann H. IL-6 modulates the synthesis of a specific set of acute phase plasma proteins in vivo. J Immunol 1989; 142:808–12.

92) de Maat MP, Pietersma A, Kofflard M, Sluiter W, Kluft C. Association of plasma fibrinogen levels with coronary artery disease, smoking and inflammatory markers. Atherosclerosis 1996; 121:185–9.

93) Powell JT. Vascular damage from smoking: disease mechanisms at the arterial wall. Vasc Med 1998; 3:21–8.

ANNEXURE I

PROFORMA

SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION AND RESEARCH

TAMAKA, KOLAR.

STUDY OF PLASMA FIBRINOGEN LEVELS IN CORONARY ARTERY DISEASE AS AN INDEPENDENT RISK FACTOR.

PROFORMA

NAME:

AGE

SEX

OCCUPATION

RELIGION

IP NO

ADDRESS

HISTORY:

LAST EPISODE OF ACUTE CORONARY EVENT:

H/O LIVER DISEASE

WHETHER PATIENT HAS FEVER

WHETHER PATIENT IS USING ORAL CONTRACEPTIVES.

PAST HISTORY:

DIABETES MELLITUS:

HYPERTENSION:

STROKE:

LIVER DISEASE:

GENERAL PHYSICAL EXAMINATION:

PALLOR

ICTERUS

CLUBBING

CYANOSIS

TEMPERATURE

EDEMA

LYMPHADENOPATHY

PULSE:

BP:

RESPIRATORY RATE

SYSTEMIC EXAMINATION

CARDIO VASCULAR SYSTEM

RESPIRATORY SYSTEM

PER ABDOMEN

CENTRAL NERVOUS SYSTEM

INVESTIGATIONS:

CBC:

ELECTROLYTES:

BLOOD UREA:

LIPID PROFILE: CHOLESTEROL:

HDL: LDL: TRIGLYCERIDES:

FBS: PPBS:

SERUM FIBRINOGEN LEVELS:

CARDIAC ENZYMES:

2D ECHOCARDIOGRAPHY:

ANGIOGRAM:

TMT:

ANNEXURE 2

INFORMED CONSENT

I, _____ unreservedly in my full sense give my consent to take part in the study, the risks and benefits of which have been explained to me in my vernacular language.

Further I do not have any objections for the presentation of this study as a part of any publication.

Signature of witness

Signature of patient/guardian

KEY TO MASTER CHART

BMI- Body Mass Index.

HDL- High Density lipoprotein

LDL- Low Density Lipoprotein.

Trop-T- Troponin T

OCP- Oral contraceptive pills

BB- Beta Blockers

ACEI- Angeiotensin converting enzyme inhibitors.

M- Male

F- Female

Y- Yes

N-No

NH- Narayana Hrudayalaya.

FBS- fasting blood sugar.

ANT- Anterior wall Myocardial infarction

INF- Inferior wall Myocardial infarction

INVESTIGATIONS

Complete blood count	
HB (gm %)	
TC(cells/mm^3)	
DC(N,L,E)	
ESR(mm/hr)	
PLATELET COUNT- lakhs/mm^3	

Lipid profile	
Cholesterol(mg/dl)	
LDL	
HDL	
Triglycerides	

Fasting sugar(mg/dl)	
Plasma fibrinogen levels(mg/dl)	