“STUDY OF TOTAL SERUM BILIRUBIN IN ACUTE CORONARY SYNDROME”

By

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Dissertation submitted to BLDE University, Vijayapur

In partial fulfilment of the requirements for the degree of

MD

IN

GENERAL MEDICINE

Under the guidance of

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2015

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ACKNOWLEDGEMENT

I have got no words to express my deep sense of gratitude and regards to my guide **Dr. Anand.P.Ambali** **M.D**, Professor of Medicine, under whose inspiring guidance & supervision, I am studying and continuing to learn the art of medicine. His deep knowledge, devotion to work and zeal of scientific research makes him a source of inspiration not only for me but for others too. It is because of his generous help, expert and vigilant supervision, that has guided & helped me to bring out this work in the present form.

My sincere thanks are due to **Dr. M S BIRADAR** **M.D** ,Principal, & **Dr. M. S. MULIMANI** Professor & HOD, Shri B.M Patil Medical College, Vijayapur, for permitting me to conduct this study.

I wish to acknowledge my Professors and take this opportunity to express my deep sense of gratitude and sincere thanks to **Dr. R.C.BIDRI, Dr. SHARAN BADIGER, Dr. S S DEVARMANI**, **Dr. L S PATIL, Dr. R M HONNUTAGI, Dr.S.N.BENTOOR, Dr.S.M.Biradar** for their supervision and timely advice.

I am also thankful for the support extended by **Dr.S.G.Balganoor, Dr.S.S.Patil, Dr. S.B.Nadagouda, Dr.G.S.Mahishale, Dr.P.G.Mantoor.**

My sincere thanks to all the staff of the Department of Biochemistry, the Department of Pathology, Shri B.M Patil Medical College Hospital & Research Centre, Vijayapur who helped me in the laboratory investigation work.

I would be failing in my duty, if I would not acknowledge my thanks to all the patients who were kind enough to help for this study.

I would also like to thank my parents **Dr Chandrakanth, Mrs. Nanda**, without their constant encouragement & moral support, my studies would have been a

distant dream. I would also like to thank my sister **Dr. Swathi** and my brother **Dr.Sachin also my best friends Mr.Sanjeev and Dr.Sandeep** for their assistance.

Finally, I would like to thank the **Almighty MAA ADISHAKTI** who gave me the energy, skill and the enthusiasm to complete this as well as the other tasks in my life & also for continuing to shower **HER** blessings upon me.

Dr. GUDAGE NITIN

LIST OF ABBREVIATIONS USED

CVD	Cardiovascular disease
CAD	Coronary Artery Disease
ACS	Acute Coronary Syndrome
ROS	Reactive Oxygen Species
TAS	Total Anti-oxidant Status
HO	Heme Oxygenase
CO	Carbon Monoxide
UGT	Uridine diphosphate Glucuronosyl Transferase
VSMC	Vascular Smooth Muscle Cell
LDL	Low Density Lipoprotein
HDL	High Density Lipoprotein
NO	Nitric Oxide
VCAM-1	Vascular Cell Adhesion Molecule-1
NF	Nuclear Factor
STEMI	ST-segment Elevation Myocardial Infarction
NSTEMI	Non–ST-segment Elevation Myocardial Infarction
UA	Unstable Angina
PCI	Percutaneous Coronary Intervention
CAC	Coronary Artery Calcification
CFR	Coronary Flow Reserve
IMT	Intima-Media Thickness
AP	Angina Pectoris
NHANES	National Health and Nutrition Examination Survey
DMSO	Di Methyl Sulf Oxide
TC	Total leucocyte Count

ABSTRACT

Background :

Atherosclerosis remains the leading cause of death and premature disability. Coronary diseases appear to result from an over balance between radical generating, compared with radical scavenging systems a condition called Oxidative stress. Bilirubin has proven to be a potent antioxidant under physiological conditions by inhibiting both lipid and protein oxidation. Reduced levels of bilirubin were shown to be associated with higher prevalence of coronary artery disease.

Objective :

To find relation between Serum Bilirubin and Acute Coronary Syndrome.

Methodology :

All patients age >18yrs, both sexes diagnosed as acute coronary syndrome based on history and relevant investigations and are admitted in ICU in BLDEU'S Shri B.M PATIL Medical college hospital and research centre Vijayapur. Total Serum Bilirubin was measured at admission and compared with standard normal mean value.

Results :

This study was conducted on 113 patients, of the study group 72.6% were male and 27.4% were female. The age ranged from 26 to 95 years of age. The mean age of the group was 54.5 ±13.19 SD. The known risk factors of ACS were studied and correlated, 37.2 % of all patients were smokers, 31% were tobacco chewers, 24.7 % had diabetes mellitus, 31.8% were hypertensive and 8 % had family history of ACS. The mean bilirubin value in mg/dl for STEMI was 0.48 ± 0.30 SD, for NSTEMI it was 0.45± 0.16 and for unstable angina it was 0.28 ± 0.1 SD. The mean total bilirubin in patients with ACS was 0.48 ± 0.28 SD mg/dl compared to bilirubin levels of 0.6mg/dl in normal population ($p<0.0001$).

Conclusion :

This study showed an inverse correlation of bilirubin with ACS. This reinforces the fact that bilirubin acts as an antioxidant and has cardioprotective action making it as emerging potential risk factor marker.

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INTRODUCTION

It is predicted that more than half the worldwide cardiovascular disease (CVD) risk burden will be borne by the Indian subcontinent in the next decade according to recent epidemiological studies and will be the largest cause of death and disability in India by 2020.[1,2,3] Coronary Artey disease (CAD) accounts for the greatest proportion of CVD[4] and Acute Coronary Syndrome (ACS) a common complication of CAD[5]. Cardiovascular risk factors for ACS are on the rise in people of Indian origin, and ACS is now the leading cause of death.[6,7,8,9,10]

Most cases of ACS are caused by rupture of an atherosclerotic plaque in a coronary artery, resulting in the formation of a thrombus.[11] Atherosclerosis appear to result from an over balance between radical generating, compared with radical scavenging systems, a condition called Oxidative stress.[12,13] Reactive Oxygen Species (ROS) can damage endothelial cells in many ways, either directly or indirectly.[14] They can also increase endothelial cell permeability and there by accelerate the accumulation of atherogenic factors, such as Low Density Lipoprotein (LDL) in the sub endothelial space.[15]

Total anti-oxidant status (TAS) in human serum reflects the balance between oxidants and antioxidants in each system. Oxidative stress has been implicated in the pathogenesis of Coronary diseases.[16,17] Increased production of ROS will result in reduced antioxidant levels.[18]

Bilirubin was considered to be a waste product of the Heme Oxygenase action but now among various benefits, it is found to have strong relation with coronary artery lesion types.[19]This is because of its major antioxidant action under physiological conditions by inhibiting both lipid and protein oxidation.[20]As little as

10nM of bilirubin is enough to protect cells against a 10000-fold higher concentration of oxidants through rapid generation of bilirubin by biliverdin reductase.[21] Additionally, bilirubin exerts anti-inflammatory effects on vasculature and inhibits proliferation of vascular smooth muscle cells.[22,23]

Hence Bilirubin was proved to act against plaque formation and subsequent atherosclerosis.[24]

The recently concluded INTERHEART study emphasized on early detection of persons with risk factors[25] and as reduced levels of bilirubin were shown to be associated with higher prevalence of coronary artery disease[26] vice versa as Individuals with Gilbert syndrome who have mildly elevated bilirubin levels were found to have ischemic heart disease rate of 2% compared with 12.1% in general population.[27] Hence making Bilirubin as emerging potential risk factor marker.

OBJECTIVE OF STUDY:

To find relation between total serum bilirubin levels and acute coronary syndrome.

REVIEW OF LITERATURE:

EPIDEMIOLOGY

World scenario :

Coronary heart disease is a worldwide health epidemic.[28] ACS a common complication of this and is associated with more than 2.5 million hospitalizations worldwide each year.[5] In the United states, for example, A conservative estimate for the number of discharges with ACS from hospitals in 2006 was 13,65000 unique for ACS.[29]

Indian scenario :

India has the highest burden of ACS in the world. Treatment and outcomes of Acute Coronary Syndromes in India (CREATE) registry has provided contemporary data on 20,468 patients with ACS from 89 centers from 10 regions and 50 cities in India and found higher 30 day mortality than developed countries.[30] Cardiovascular risk factors for ACS are on the rise in people of Indian origin and ACS is now the leading cause of death.[6,7,8,9,10]

Realtion of serum Bilirubin with ACS

Introduction

For many years, the bile pigment bilirubin was considered a toxic waste product formed during heme catabolism. However more recent evidence suggests that bilirubin is a potent physiological antioxidant that may provide important protection against atherosclerosis and inflammation. It is generally accepted that oxidative reactions are involved in the pathophysiology of these disease processes. Substantial

evidence has documented that the development of CAD involves lipid oxidation and formation of oxygen radicals as atherosclerosis and inflammation are associated with formation of oxygen and peroxyl radicals.[12-17,19,22-24,37-39,41-58,60]

In 1987, Stocker R, Yamamoto Y, McDonagh AF, Glazer AN, Ames BN[24] for the first time demonstrated that bilirubin scavenged peroxyl radicals more effectively than did the powerful antioxidant α-tocopherol in vitro. Since then, several investigators have shown that bilirubin has antioxidant effects, including inhibition of membrane lipid peroxidation and scavenging of ROS[31-38].This has led to suggestions that mildly increased circulatory bilirubin may have a physiologic function to protect against disease processes that involve oxygen and peroxyl radicals or vice versa and many studies have shown relation with CAD.[39-43]

Serum bilirubin : Synthesis of Bilirubin and metabolism

Bilirubin (formerly referred to as hematoidin) is the end product of haem, the majority (80–85%) coming from haemoglobin (a principal component of red blood cells) with only a small fraction derived from other haem-containing proteins such as cytochrome P450. Approximately 300mg bilirubin is formed daily. Production from haemoglobin takes place in reticulo-endothelial cells.The enzyme that converts haem to bilirubin is microsomal haem oxygenase (Figure 1).[44]

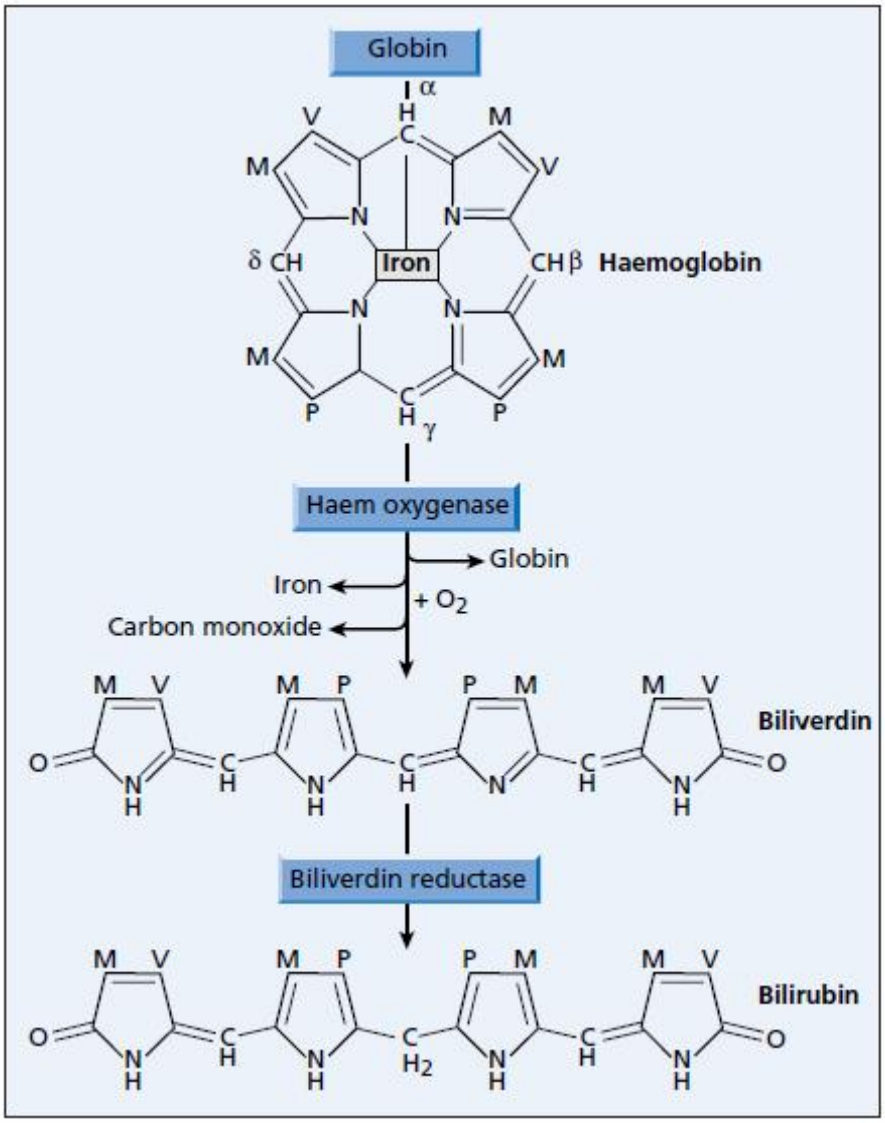

Figure 1[44]-The metabolism of haemoglobin to bilirubin. M,methyl; P, propionate; V, vinyl

Heme oxygenase (HO) is the rate-limiting enzyme of bilirubin production. It is a microsomal enzyme present in both central and peripheral tissues, that converts heme to Biliverdin and Carbon monoxide (CO). Biliverdin is subsequently reduced to bilirubin by the cytosolic enzyme biliverdin reductase.[44]

HO causes Cleavage of the porphyrin ring selectively at the a-methane bridge. The a-bridge carbon atoms converted to carbon monoxide and the original bridge

function is replaced by two oxygen atoms which are derived from molecular oxygen. The resulting linear tetrapyrrole has the structure of the IX a-biliverdin. This is converted further to IX a-bilirubin by a cytosolic enzyme, biliverdin reductase, Such a linear tetrapyrrole should be water soluble, where as bilirubin is lipid soluble. The lipid solubility is explained by realignment of the pyrole ring such that internal hydrogen bonding masks the propionic acid side chains making bilirubin poorly soluble in aqueous solvents. This bonding can be broken by alcohol in the diazo (van den Bergh) reaction converting unconjugated, indirect bilirubin to direct reacting bilirubin. In vivo the stable hydrogen bonds are altered by esterification of the propionic groups by glucuronic acid.[44]

About 20% of circulating bilirubin is not formed from the haem of mature erythrocytes. A small proportion comes from immature cells in the spleen and bone marrow. This component is increased in haemolytic states.The remainder is formed in the liver from haem proteins such as myoglobin, cytochromes and unknown sources.[44]

Hepatic transport and conjugation of bilirubin[44](Figure 2)[44]

Unconjugated bilirubin is transported in the plasma tightly bound to albumin. A very small amount is dialysable, but this can be increased by substances such as fatty acids and organic anions which compete with bilirubin for albumin binding.

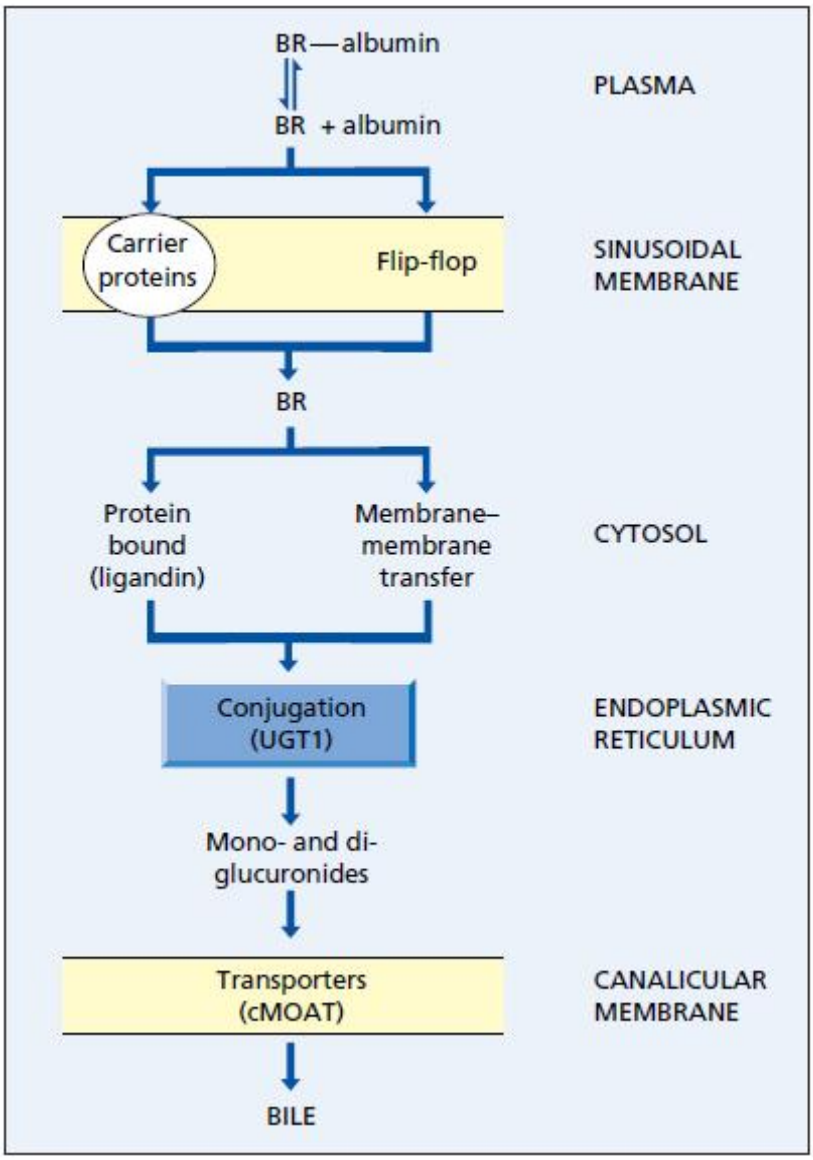

Figure 2[44]-Bilirubin (BR) uptake, metabolism and secretion by the hepatocyte. MOAT, multi-specific organic anion transporter; UGT1, uridine diphosphate glucuronosyl transferase-1.

The liver extracts organic anions including fatty acids and bile acid and non-bile acid cholephils, such as bilirubin, despite tight albumin binding. Studies suggest that bilirubin dissociating from albumin in the sinusoid diffuses across the unstirred water layer at the surface of the hepatocyte. The mechanism for passage of bilirubin across the plasma membrane into the hepatocyte involves either transport proteins

such as the organic anion transporter and/or bilirubin flip-flop across the membrane. Uptake is highly effective because of the rapid hepatic metabolism by glucuronidization and excretion into bile, also because of binding by carrier proteins in the cytosol such as glutathione-*S*-transferase (ligandin). Unconjugated bilirubin is non-polar (lipid soluble). It is converted to a polar (water soluble) compound by conjugation and this allows its excretion into the bile. The microsomal enzyme responsible is uridine diphosphate glucuronosyl transferase (UGT) which converts unconjugated bilirubin to conjugated bilirubin mono and diglucuronide. Bilirubin UGT is a one of several UGT enzyme isoforms that are responsible for the conjugation of many endogenous metabolites, hormones and neurotransmitters.The gene expressing bilirubin UGT is on chromosome 2. The structure of the gene is complex (Figure 3)[44].

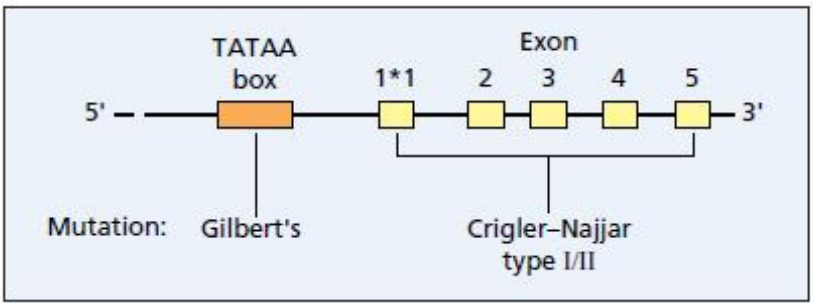

Figure 3[44] Structure of gene for bilirubin UGT1*1 with five exons and the promoter region (TATAAbox). There are several other possible first exons (not shown) that can be spliced to exons 2–5, and have other substrate specificities.

The major bilirubin conjugate in human bile is the diglucuronide. A single microsomal glucuronyl system catalyses both the conversion of bilirubin to the monoglucuronide and diglucuronide. With a high bilirubin load, as in haemolysis,

monoglucuronide formation is favoured, whereas if the bilirubin load is low, the diglucuronide increases.[44]

Although conjugation as a glucuronide remains the most important mechanism, sulphate, xylose and glucose conjugation also occur to a small extent and may be increased in cholestasis.[44]

Biliary canalicular excretion of bilirubin is mediated by the Adenosine Tri Phosphate (ATP)-dependent multi-specific organic anion transporter (cMOAT) also called multi-drug resistance protein-2 (MRP-2). Biliary excretion of glucuronide is the rate-limiting factor in the transport of bilirubin from plasma to bile. Bile acids are secreted into bile by the bile salt export pump (BSEP). The separate mechanism for bilirubin and bile acid is exemplified by the Dubin–Johnson syndrome where there is a defect in the excretion of conjugated bilirubin, while bile salt excretion is usually normal. A high proportion of the conjugated bilirubin in bile is in corporated into mixed micelles with cholesterol, phospholipids and bile salts.[44]

Bilirubin diglucuronide in bile is polar (water soluble) and hence is not absorbed from the small intestine. In the colon, bacterial b-glucuronidases hydrolyse the conjugated bilirubin, which is then reduced to urobilinogens and urobilin which are excreted in the stool (Figure 4)[44]

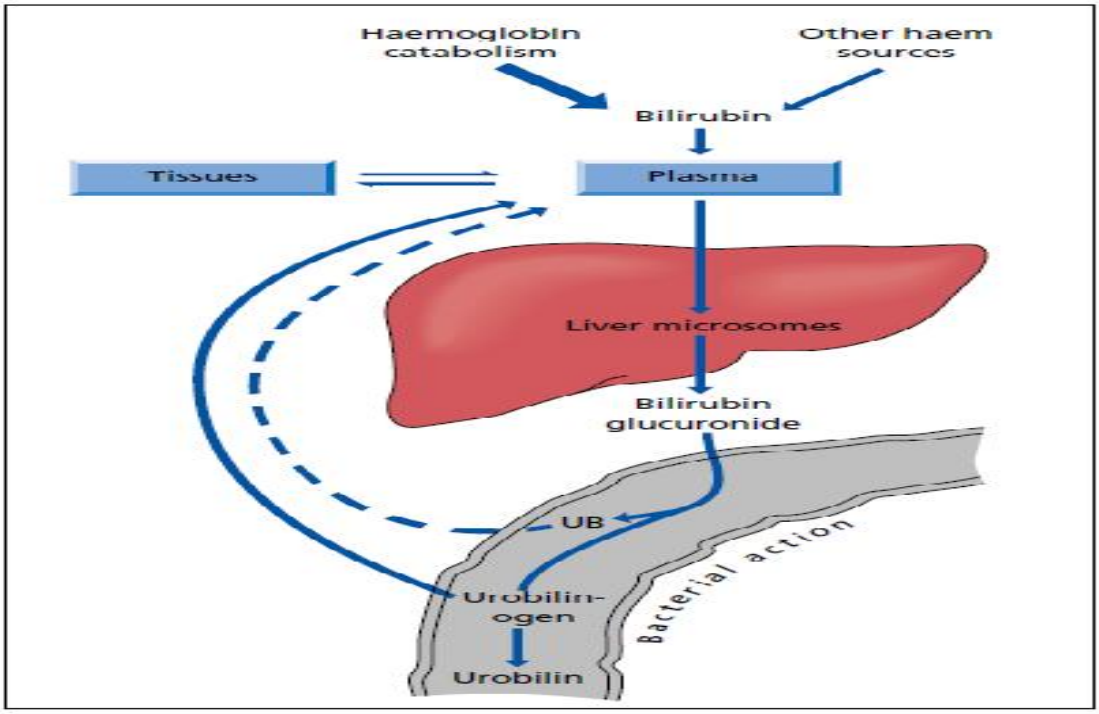

Figure 4[44]The metabolism of bilirubin. UB, unconjugated bilirubin.

Urobilinogen is non-polar and is well absorbed from the small intestine, but only minimally from the colon. The little that is normally absorbed is re-excreted by the liver (entero-hepatic circulation) and kidneys.

Pathophysiology of ACS[45,46]

The following processes that leads to ACS are not mutually exclusive and can occur simultaneously in any combination.

1.Initiation of Atherosclerosis in relation to Bilirubin

2.Thrombosis and Atheroma Complications

3.Coronary arterial vasoconstriction

4.Imbalance between the supply and demand of the myocardium for oxygen

1.Initiation of Atherosclerosis

1.1 Role of Reactive oxygen species

Atherosclerosis remains the leading cause of death and premature disability in most of population of world.[47] Age, gender, obesity, cigarette smoking, hypertension, diabetes mellitus and dyslipidemias are known atherogenic risk factors that promote the impairment of endothelial function, smooth muscle function and vessel wall metabolism. These risk factors are associated with an increased production of free radicals that are called Reactive oxygen species (ROS).[48] O2-, OH, and H2O2 that are produced during the metabolism of oxygen. These 3 species, together with unstable intermediates in the peroxidation of lipids, are referred to as ROS.[49] (Table 1)[50] Atherosclerosis appear to result from an over balance between radical generating, compared with radical scavenging systems, a condition called Oxidative stress.[12,13] During the last decades several studies have examined the potential role of oxidative stress in atherogenesis.[51,52]

In the vascular wall, ROS are produced by all layers, including endothelium, smooth muscle and endothelia.[53] Under physiological conditions ROS are produced in low concentrations and act as signalling molecule that regulate vascular smooth muscle cell (VSMC) contraction and relaxation, and participate in VSMC growth.[54,55]

Under pathological conditions these free radicals play role in atherosclerosis [14,56-58]

Table 1[50]. Examples of free radicals in biological systems.

Name	Formula
Superoxide anions	$O2^-$
Alkoxyl and peroxyl radical	RO, RO2
Hydroxyl radica	OH
Nitric oxide and nitrogen dioxide	NO, NO2

A number of mechanisms, which are not mutually exclusive, have been proposed to explain a possible connection between oxidative stress and coronary artery disease.

Bilirubin Is a Physiologic Antioxidant

Athersosclerosis results from oxidation stresss[12,13,48,51,52] The most straight forward explanation for the observed protection of bilirubin against CVD is the antioxidant capacity of Bilirubin that has been known for many years.[24]

In several studies[20,21] it was found that different circulating forms of bilirubin are powerful antioxidants: The proposed mechanism is[24] :

Bilirubin can scavenge the chain-carrying peroxyl radical by donating a hydrogen atom attached to the C-10 bridge of the tetrapyrrole molecule to form a carbon-centered radical Bil•

LOO• + Bil = LOOH + Bil•

Bil• + LOO• = Bil-OOL

Bil• + O2 =Bil-OO•

LOO• + BV = LOO-BV

Free bilirubin, albumin-bound bilirubin, conjugated bilirubin, and unconjugated bilirubin were all noted to be effective scavengers of peroxyl radicals(Figure 5)[59]

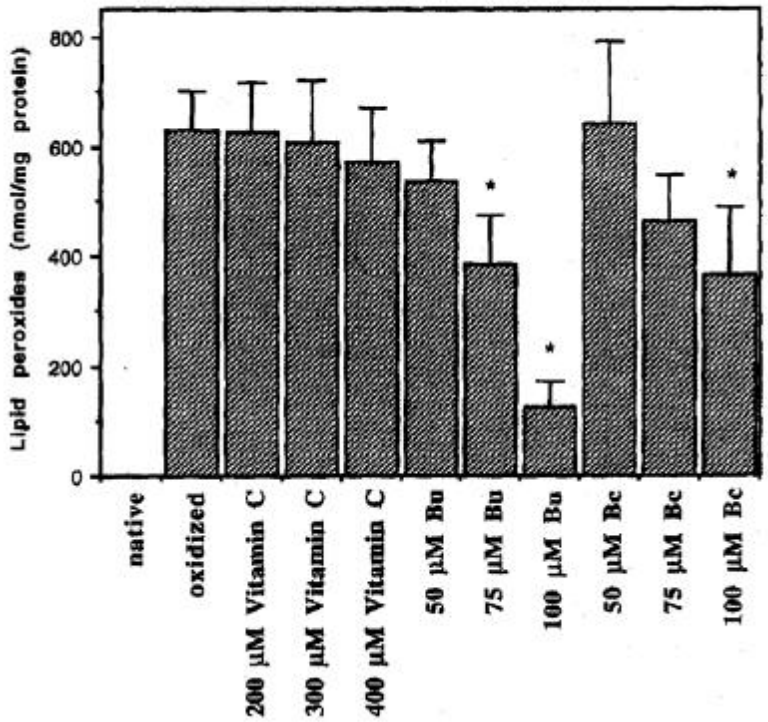

Figure 5[59] Both unconjugated bilirubin (Bu) and conjugated bilirubin (Bc) can serve as antioxidants, protecting human LDL from lipid peroxidation in vitro against peroxyl radicals.

In a human study, Benitez et al.[60] found that increase in the concentration of uric acid, bilirubin, and ascorbic acid after aerobic exercise in 11 male athletes result in a significant increase in total antioxidant serum capacity measured by reverse-phase chromatography which can measure the oxidation of LDL and HDL (High Density Lipoprotein) cholesterol.

The reduced bilirubin levels in smokers clearly demonstrate the potential impact of oxidative stress. This is underscored by the association between serum and urine levels of oxidative metabolites of bilirubin with mortality in acute myocardial infarction.[61]

Reactive oxygen species have detrimental effects on vascular function through several mechanisms.[48] (Figure 6)[48]

1.2 On Endothelium and vascular smooth muscle cells

ROS can damage endothelial cells in many ways, either directly or indirectly.[14] ROS can promote endothelial apoptosis, leading to an increased tendency toward thrombosis and endothelial dysfunction.[62] Evidences have shown endothelial dysfunction a strong predictor of cardiovascular dysfunction in patients with cardiovascular risk factors.[62,63] Endothelial dysfunction leads to rapid decrease in Nitric oxide (NO) production or availability, partially due to inactivation of NO by superoxide.[50]

In stages of advanced atherosclerosis, despite the fact that NO production remains the same, decomposition of NO from ROS is increased.[64] Decreased concentration of NO leads to vasoconstriction, platelet aggregation and adhesion of neutrophils to the endothelium.[64]

ROS can also increase endothelial permeability and thereby accelerate the accumulation of athrogenic factors, such as LDL in the sub endothelial cell space.[12]

Bilirubin inhibits endothelial dysfunction. It has been reported to prevent endothelial cell sloughing caused by hyperglycaemia. In an animal model of streptozotocin-induced diabetes, administration of bilirubin prevented hyperglycaemia

induced endothelial cell sloughing via a decrease in oxidative stress. These results demonstrate that bilirubin may be a novel approach to prophylactic vascular protection in diabetes.[65] Treatment of endothelial cells with HOCl (Hypochlorous acid) stimulated mitochondrial dysfunction, caspase-3 activation and cell death administration of bilirubin reversed these actions.[66] These results suggest that bilirubin may represent a critical adaptive response to maintain endothelial cell viability at sites of vascular inflammation and atherosclerosis.[67] In a human study Yoshino et al.[68] investigated patients without coronary heart disease who underwent coronary flow studies. Coronary dilatation following papaverine injection during coronary angiography was measured to assess the effects of bilirubin on endothelial function. In this study log-transformed total bilirubin is positively correlated with flow-mediated dilatation, suggesting that a high bilirubin level is associated with favourable coronary endothelial function.

1.3 Lipid metabolism

ROS peroxidize lipid components, leading to the formation of oxidized lipoproteins (LDL), one of the key mediators of atherosclerosis. Whereas naive LDL does not cause cholesterol ester accumulation in macrophages, LDL modified by oxidation does.[48]

Since the first proposal of the LDL oxidation hypothesis for atherosclerosis by Steinberg and his colleagues in 1989,[69] Ample evidence has been presented supporting the hypothesis that oxidative modification of LDL is the key initial event for the progression of atherosclerosis.[70,71]

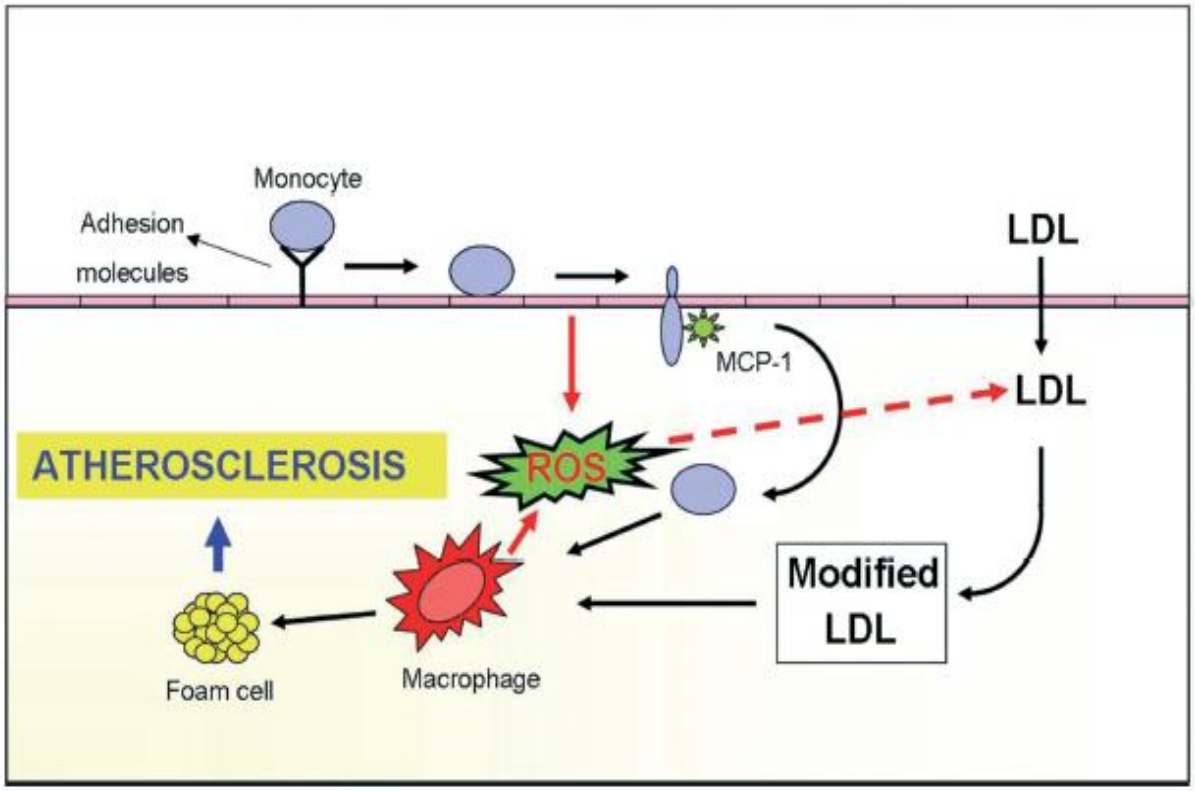

Figure 6.[48] Oxidative stress in atherosclerosis. radicals provoke an oxidative modification from low-density lipoprotein (LDL) to oxidized low-density lipoprotein (oxLDL). Circulating monocytes migration to the subendothelial space is stimulated by ox-LDL and also causes endothelial cell injury. The modified LDL is taken up by macrophages which become foam cells, leading to the formation of atherosclerotic plaque.

1.4 Bilirubin-mediated inhibition of lipid oxidation

Bilirubin has also been shown to have powerful antioxidant activity against oxidation of lipoproteins, which is one of the important steps of plaque formation and atherosclerosis. Indeed, bilirubin has been demonstrated to be almost 30 times more potent towards the prevention of LDL oxidation compared to a vitamin E analogue, Trolox, which represents a lipid-soluble antioxidant substance.[72] It has also been noted that bilirubin is more effective at protecting lipids from oxidation than

the water-soluble antioxidants such as glutathione, which primarily protects proteins from oxidation.[37]

It was suggested bilirubin to serve as a potent lipid chain-breaking antioxidant under physiological conditions and to able to protect human LDL against peroxidation[31-38] and increased physiological concentrations of plasma bilirubin may reduce atherogenic risk or vice versa[27,39-43]

It is now clear that oxidation of LDL lipids and apolipoprotein B 100 renders LDL Pro-atherogenic[73] and furthermore HDL oxidation impairs its inherent anti-atherogenic properties[74] also its anti-inflammatory function.[75]

Bilirubin may also affect composition of lipoproteins. One study has revealed that bilirubin is significantly correlated with apolipoprotein B, which is a better predictor than LDL cholesterol level.[76] Upon receiver operator characteristic curve (ROC) analysis, bilirubin showed an inverse relationship with risk of CAD, with the area under the ROC curve comparable to lipoprotein.[76] O' Kane et al.[77] compared serum lipids and the apolipoprotein levels of patients with primary biliary cirrhosis with those of a normal control, the level of apolipoprotein A1, which protects against atherosclerosis is higher in the elevated bilirubin group. Serum Amyloid A (SAA) proteins, a family of apolipoproteins associated with HDL, are produced by the liver during the acute phase of inflammation. They are implicated in several chronic inflammatory diseases, including atherosclerosis.[78] Higher SAA levels impair HDL antioxidative functionality.[79] Bilirubin was correlated inversely with SAA levels in subjects without metabolic syndrome.[80] These raise the possibility that metabolic syndrome may elicit abnormalities in HDL antioxidative function, which could mask a relationship between SAA and bilirubin. This was supported by the fact that of the

individual metabolic syndrome components the strongest effect, modification of bilirubin on SAA, was observed for HDL cholesterol. Remnant-like particle (RLP) cholesterol is an independent cardiovascular disease risk factor in women according to the results from the Framingham heart study.[81] The relationship between serum bilirubin level and serum RLP cholesterol was investigated in 270 males who visited the outpatient clinic. It was found that the serum RLP cholesterol level was significantly lower in patients in the high bilirubin group than in those in the lower bilirubin group. These findings suggest that serum bilirubin with in physiological levels may affect the serum RLP cholesterol and may play a role in preventing atherosclerosis. It is not clear however how bilirubin makes these changes of lipoproteins.

1.5 Bilirubin as reflection of enhanced HO activity

Heme oxygenase (HO) the rate-limiting enzyme of bilirubin production At least two isoforms of HO have been identified and found to be products of different genes and to differ in their tissue expression, function and ability to respond to stimuli. HO-1 ($M_r \cong 32\ 000$) is an inducible form that is expressed at a low concentration in vascular endothelial and smooth muscle cells and is markedly induced by heme, metals, oxidative stress, inflammatory mediators, oxidized LDL and hypoxia. A variety of experiments have suggested that HO-1 is a stress-response protein that plays an important function in cell defense mechanisms against oxidative injury.[82] HO-1 activity is responsible for increased Carbon monoxide and bilirubin formation as well as iron release in pathological conditions such as CAD.[83-87]

Another isoform of heme oxygenase is a constitutive enzyme (HO-2; $M_r \cong 34\ 000$) which produces biliverdin and CO under normal physiological conditions[82]

HO-1-mediated consumption of heme may reduce heme-induced toxic cell injury and decreased hemoglobin concentrations may enhance vasodilatation. Furthermore, hemoglobin is a scavenger of NO that blunts NO-dependent vasodilatation. CO could affect cardiovascular function through activation of soluble guanylate cyclase and the consequent increase in intracellular cGMP concentrations[82]. CO is also an active vasodilator involved in the regulation of vasomotor tone, platelet aggregation, and vascular smooth muscle cell proliferation.[82,87,88]

Increased HO activity may account for the antiatherogenic and cardioprotective effects of bilirubin through increased elimination of heme and/or enhanced production of CO, iron, and biliverdin. Changes in the concentration of any of these metabolites could affect the pathophysiology of atherosclerosis.[82-86](Figure 7)[87]

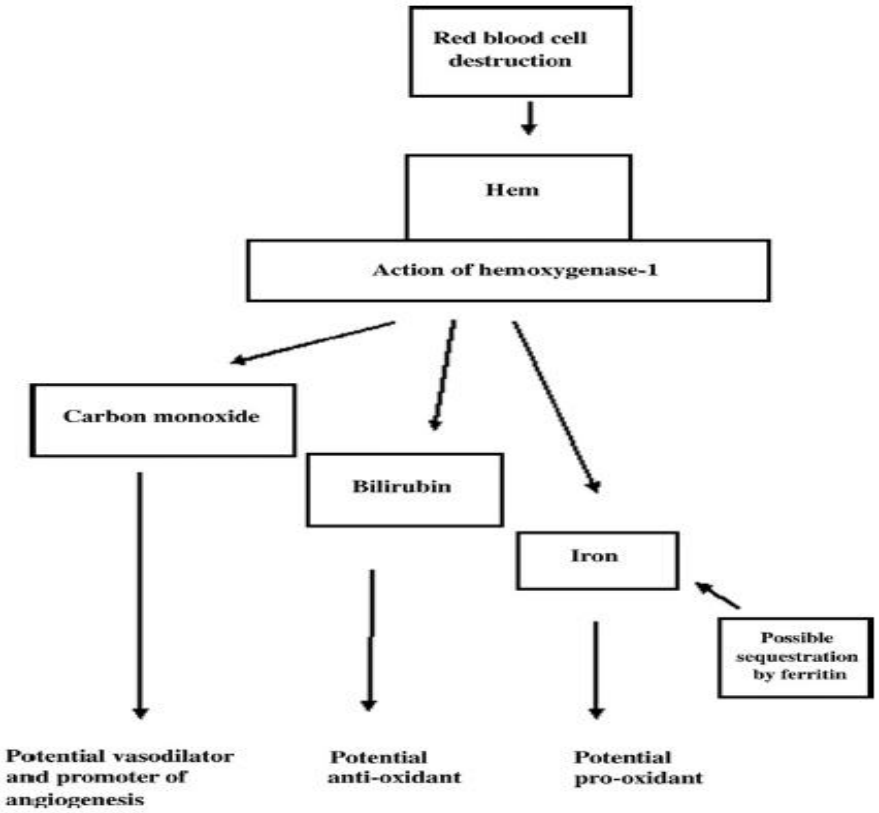

Figure 7[87]- Hem released from red blood cells is enzymatically converted into carbon monoxide, bilirubin, and iron. The former is a vasodilator and promoter of angiogenesis. Bilirubin has potential antioxidant activity.

1.6 Role of inflammation

Once the endothelium has been damaged, the inflammatory cells, especially monocytes migrate into the subendothelium by binding to endothelial adhesion molecules; once in the sub endothelium, they undergo differentiation, becoming macrophages. Macrophages digest oxidized LDL that has also penetrated the arterial wall, transforming into foam cells i.e. lipid-laden macrophages characteristic of early-stage atherosclerosis and causing the formation of fatty streaks.[89,90]

Hence two important steps for atherosclerosis are oxidative stress and inflammation (Figure 8 and 9)[91]

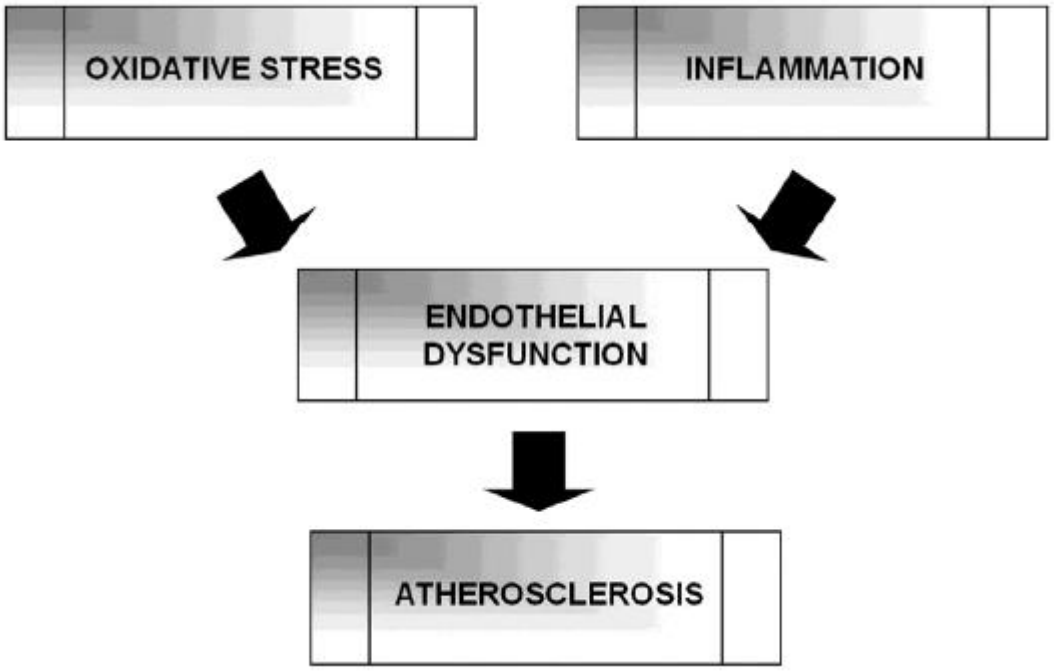

Figure 8.[91] **Role of Oxidative stress and inflammation in atherosclerosis.**

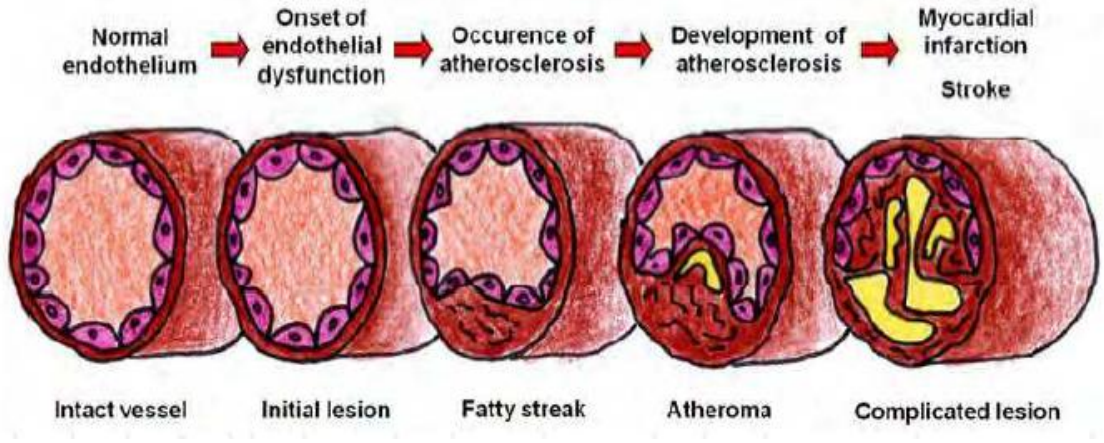

Figure 9.[91] **Steps involved in atherosclerotic progression**

1.7 Bilirubin as anti inflammatory

An involvement of bilirubin in immune reactions and inflammatory processes has also been documented. A correlation between bilirubin metabolism and inflammatory processes is also supported by observations that high HO activity is linked to a faster resolution of inflammation whereas inhibition of this enzyme appears to potentiate the inflammatory responses[92]. Both endothelial and inflammatory cells[93] express all of the necessary enzymes involved in bilirubin synthesis and degradation, implies a high degree of regulation of cellular bilirubin levels and supports a potentially broad role for this endogenous bile pigment as a physiological regulator of inflammation. Serum Bilirubin is known to inhibit the inflammatory response.[94]

Ishikawa et al.[95] have reported that inducible HO-1 is highly induced by oxidized LDL. This augmented HO-1 induction resulted in the reduction of monocyte chemotaxis in response to LDL oxidation. Furthermore pretreatment with bilirubin, the product of HO, further reduced chemotaxis of monocytes. Similarly, chemotactic activity stimulated by angiotensin II was suppressed by HO activity and accumulation

of bilirubin in monocytes. Notably exogenously applied bilirubin and carbon monoxide mimicked the inhibitory effect of HO-1 on the chemotactic response.[96]

In addition, it has been suggested that bilirubin and HO-1 simultaneously affect the activity of macrophages. Expression of HO-1 induction and bilirubin IX α production in foam cells in atherosclerotic regions of hypercholesterolemic rabbits was proved by in situ hybridization and immunohistochemistry. These results provide the first in vivo evidence of the colocalization of HO-1 and bilirubin IX α in foam cells suggesting a role of HO-1 induction and bilirubin in the modulation of macrophage activation in atherosclerosis.[97]

Both endothelial and inflammatory cells express all of the necessary enzymes involved in VCAM-1-(Vascular cell adhesion molecule-1) mediated leukocyte recruitment is implicated in the pathogenesis of a number of inflammatory conditions including atherosclerosis[98] and inflammatory bowel disease.[99,100] With regard to the former, bilirubin synthesis and degradation implies a high degree of regulation of cellular bilirubin levels and supports a potentially broad role for this endogenous bile pigment as a physiological regulator of inflammation VCAM-1 is detectable in atherosclerotic plaques and endothelial expression of this adhesion molecule has been shown to be an early event at sites predisposed to atherosclerosis. Moreover, disruption of VCAM-1 expression in the atherosclerosis prone low density lipoprotein receptor knockout mouse is associated with a significant decrease in the number of vascular lesions. Consistent with the hypothesis that bilirubin may modulate the process of atherogenesis by inhibiting VCAM-1 signaling a host of epidemiological analyses have identified an inverse correlation between serum bilirubin levels and both the risk and severity of cardiovascular disease.[101]

Bilirubin has also been shown to significantly interfere with the complement

system, with all of the possible protective consequences[102]. Consistent with this data, several studies have demonstrated a negative relationship between serum bilirubin and C-reactive protein levels[94,103,104]. In addition, the modulatory effects of bilirubin on T regulatory cell differentiation were recently reported[105] further underlining the protective role of bilirubin in the pathogenesis of chronic inflammatory as well as in autoimmune conditions.

There is also much evidence that bilirubin affects cell signal transductions. Bilirubin has prevented the nuclear translocation of nuclear factor (NF)-kappaB induced by TNF- α. By inhibition of the NF-kappaB transduction pathway, bilirubin blunted the over expression of adhesion molecules in endothelial cells. Collectively these data may contribute to explain the protective effect of bilirubin against development of atherosclerosis.[106]

Other mechanisms

Bilirubin inhibits smooth muscle cell proliferation:

In addition to influencing the inflammatory processes, bilirubin can prevent neointimal formation by inhibiting the proliferation of smooth muscle cells. For example, bilirubin has been shown to attenuate balloon injury-induced neointimal formation in Gunn rat and in wild-type rat models.[107] In vitro bilirubin and biliverdin have inhibited serum-driven smooth muscle cell cycle progression at the G1 phase via inhibition of the mitogen-activated protein kinase signal transduction pathways and inhibition of phosphorylation of the retinoblastoma tumour suppressor protein.[107] The same authors also demonstrated that bilirubin and biliverdin caused p53-dependent cell cycle arrest by hypo phosphorylation of the retinoblastoma tumour suppressor protein in growth factor-stimulated vascular smooth muscle cells in a balloon injury

model in rats[108]. Another study has shown that local administration of bilirubin attenuates neointimal formation following injury of rat carotid arteries and regulates the proliferation and migration of human arterial smooth muscle cells.[109] In this study bilirubin inhibits neointimal formation after arterial injury, and this is associated with alteration in the expression of cell cycle regulatory proteins. Furthermore, bilirubin blocks proliferation and migration of human arterial smooth muscle cells and arrests smooth muscle cells in the G0/G1 phase of the cell cycle.[109] It is also known that bilirubin affects other cell signalling pathways to inhibit smooth muscle cell proliferation. Stoeckius et al.[110] further investigated the molecular events by which bilirubin inhibits growth of proliferation of human coronary artery smooth muscle cells. In this study bilirubin impaired the activation of the Raf/ERK/MAPK pathway and the cellular Raf and cyclin D1 content that results in retinoblastoma protein hypo phosphorylation on amino acids S608 and S780. These events impede the release of YY1 to the nuclei and calcium dependent YY1 proteolysis in human vascular cells. They concluded that in the serum-stimulated human vascular smooth muscle primary cell cultures bilirubin favours growth arrest and proposed that this activity is regulated by its interaction with the Raf/ERK/MAPK pathway, the effect on cyclin D1 and Raf content, the altered retinoblastoma protein profile of hypo phosphorylation, calcium influx, and YY1 proteolysis. These observations provide important mechanistic insight into the molecular mechanism underlying the transition of human vascular smooth muscle cells from proliferative to contractile phenotype and the role of bilirubin in this transition.[110] These inhibitory effects of bilirubin of neointimal formation have also been demonstrated in a human study. The main mechanism of in-stent restenosis (ISR) is known as neointimal formation. Kuwano et al[111]. investigated 1076 consecutive patients who underwent coronary stenting and follow-up

angiography. In this study the ISR rate at follow-up is significantly correlated with the total bilirubin level. A significant negative correlation between the total bilirubin and ISR was revealed by multivariate analysis (OR 0.6; 95% CI 0.39 – 0.89).

Another possibility is that low bilirubin concentrations per se are not a major causative factor in the development of CAD, but rather a reflection of the presence of this ailment. According to this view, low bilirubin is a result of increased oxidative activity in CAD-prone individuals, leading to consumption of natural antioxidants.[112]

Although the mechanisms underlying the ability of certain bilirubin concentrations to protect against CAD remain to be clarified, it is possible that either the bilirubin concentration itself or changes in the concentrations of other components in the bilirubin synthetic pathway are involved in the protective action. These additional components may include heme, biliverdin, CO and iron which are regulated by the activity of HO and have all been implicated in the physiology and pathology of the cardiovascular system. HO is expressed at low basal concentrations in vascular endothelial and smooth muscle cells and is induced by oxidative stress, inflammatory mediators and oxidized LDL. The complex interactions between HO expression the circulating concentrations of its substrate and products and the effect of these components and specifically of bilirubin, on the vasculature, on lipid metabolism and on the cardiovascular system will hopefully be the focus of extensive research in the coming years.[112]

In conclusion follwoing mechanisms expalin cardioprotective action of bilirubin(FIGURE 10)[113]

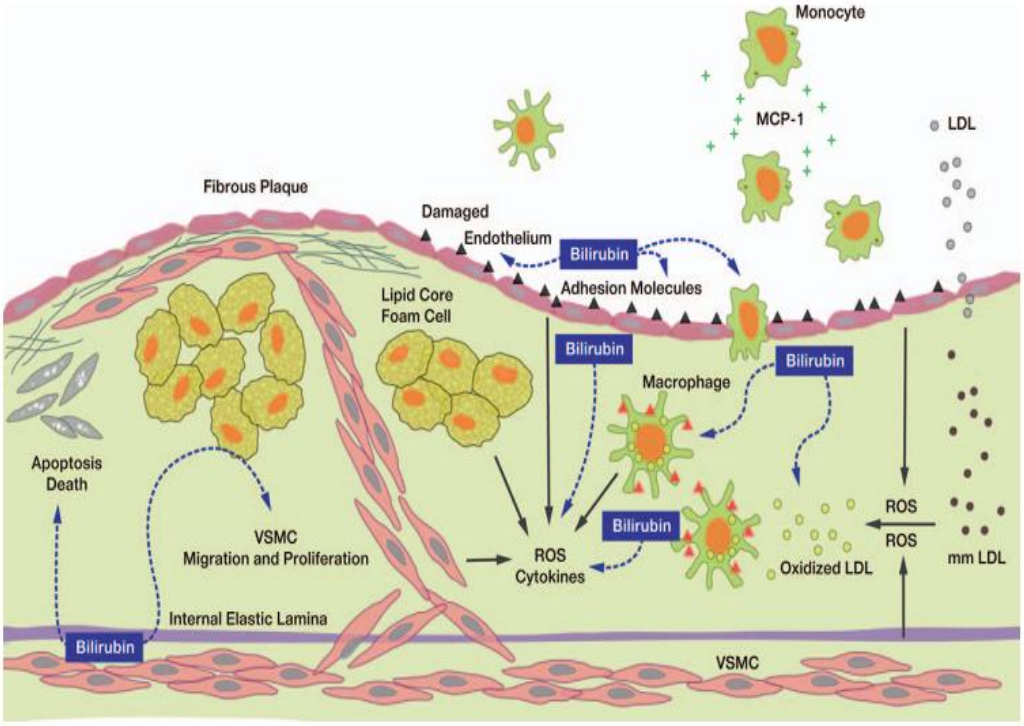

Figure 10[113]. Protective role of bilirubin against atherosclerosis. Oxidation of LDL and ROS itself involves atherosclerosis progression. Bilirubin has antioxidant properties, inhibits monocyte chemotaxis, attenuates expression of adhesion molecules on endothelial cells, improves endothelial dysfunction and inhibits proliferation of VSMCs. Protective properties of bilirubin in the atherosclerotic processes are indicated as blue dotted arrows. LDL lowdensity lipoprotein; MCP-1 monocyte chemotactic protein-1; ROS reactive oxygen species; VSMC vascular smooth muscle cell.

2. Thrombosis and Atheroma Complications

Several major modes of plaque disruption provoke most coronary thrombi.[114-116] The first mechanism, accounting for some two thirds of acute myocardial infarctions involves a fracture of the plaque's fibrous cap. Another mode involves a superficial erosion of the intima, accounting for at least a quarter of acute myocardial infarctions in selected referral cases of sudden cardiac death.[117]

Following plaque rupture/erosion activation of the coagulation cascade and platelets plays a central role in the formation of thrombus. The first step in thrombus formation is vascular injury or endothelial dysfunction, which causes adhesion of platelets to the arterial wall via binding of platelet glycoprotein (GP) Ib to

subendothelial von Willebrand factor. Exposure of platelets to subendothelial collagen and/or circulating thrombin causes platelet *activation*, which induces platelets to change shape and results in degranulation with release of adenosine diphosphate (ADP) and thromboxane A2 (TxA2)—which in turn causes further platelet activation and expression of platelet glycoprotein GP IIb/IIIa.

In parallel, tissue factor expressed within the lipid-rich core of atherosclerotic plaque, when exposed to circulating blood, activates the coagulation cascade. A complex of tissue factor and coagulation factors VIIa and Va leads to the formation of activated factor X (factor Xa), which in turn amplifies the production of activated factor IIa (thrombin). The cascade proceeds with thrombin-induced conversion of fibrinogen to fibrin. The platelet and coagulation systems converge in that thrombin is also a potent platelet activator. Platelet GP IIb/IIIa binds circulating fibrinogen, thereby causing platelet aggregation and ultimately producing a platelet-fibrin thrombus, portions of which may embolize distally and cause myocardial necrosis.

3**)Coronary arterial vasoconstriction**[46]

Vasoconstriction causing dynamic obstruction of coronary arterial flow may result from spasm of the epicardial coronary arteries (Prinzmetal angina)—constriction of small intramural, muscular coronary arteries resulting in increased coronary vascular resistance. This constriction may result from vasoconstrictors released by platelets, endothelial dysfunction or adrenergic stimuli (e.g., the “fight-or-flight” response, cold, cocaine, or amphetamines). More than one of these mechanisms may be present simultaneously.

4) Imbalance between the supply and demand of the myocardium for oxygen[46,118]

In normal conditions, for any given level of a demand for oxygen, the myocardium will control the supply of oxygen-rich blood to prevent underperfusion of myocytes and the subsequent development of ischemia and infarction. The major determinants of myocardial oxygen demand (MVO_2) are heart rate, myocardial contractility and myocardial wall tension (stress).

By reducing the lumen of the coronary arteries, atherosclerosis limits appropriate increases in perfusion when the demand for flow is augmented, as occurs during exertion or excitement. When the luminal reduction is severe, myocardial perfusion in the basal state is reduced.

Myocardial ischemia also can occur if myocardial oxygen demands are markedly increased and particularly when coronary blood flow may be limited, as occurs in severe left ventricular hypertrophy due to aortic stenosis. The latter can present with angina that is indistinguishable from that caused by coronary atherosclerosis largely owing to subendocardial ischemia. A reduction in the oxygen-carrying capacity of the blood as in extremely severe anemia or in the presence of carboxyhemoglobin and hypotension rarely causes myocardial ischemia by itself but may lower the threshold for ischemia in patients with moderate coronary obstruction.

When an increase in myocardial O2 demand (e.g., tachycardia, fever, thyrotoxicosis) occurs in a patient with fixed narrowing of an epicardial coronary artery, secondary NSTE-ACS may develop.

Not infrequently, two or more causes of ischemia coexist in a patient, such as an increase in oxygen demand due to left ventricular hypertrophy secondary to

hypertension and a reduction in oxygen supply secondary to coronary atherosclerosis and anemia. Abnormal constriction or failure of normal dilation of the coronary resistance vessels also can cause ischemia. When it causes angina, this condition is referred to as *microvascular angina*

All the above mentioned pathophysiology results in Ischemic Heart Disease

Ischemic heart disease (IHD) is a condition in which there is an inadequate supply of blood and oxygen to a portion of the myocardium; it typically occurs when there is an imbalance between myocardial oxygen supply and demand. The most common cause of myocardial ischemia is atherosclerotic disease of an epicardial coronary artery (or arteries) sufficient to cause a regional reduction in myocardial blood flow and inadequate perfusion of the myocardium supplied by the involved coronary artery.[118]

Ischemic heart disease may be manifested clinically as either chronic stable angina or an acute coronary syndrome (ACS).

Features that help differentiate ACS from stable angina are (1) onset of symptoms at rest (or with minimal exertion) and lasting longer than 10 minutes unless treated promptly; (2) severe, oppressive pressure or chest discomfort; and (3) an accelerating pattern of symptoms that develop more frequently, occur with greater severity, or awaken the patient from sleep.[46] ACS can be subdivided into ST-segment elevation myocardial infarction (STEMI), non–ST-segment elevation myocardial infarction (NSTEMI), or unstable angina (UA).

Because NSTEMI and UA are indistinguishable at initial evaluation and the entity of UA is receding as the sensitivity of biomarkers of myocardial injury

increases, they are often described together as NSTE-ACS, symptoms alone do not suffice to distinguish the three types of ACS from one another. Patients without persistent (>20 minutes) ST-segment elevation in two or more contiguous leads but with biomarker evidence of myocardial necrosis are classified as having NSTEMI, whereas in patients without such evidence of myocardial necrosis, UA is diagnosed—a condition generally carrying a better prognosis.[46]

THERAPEUTIC GOALS AND APPROACHES FOR ACS[119,120]

Initial assessment:

Consider the diagnosis in patients with chest discomfort, shortness of breath, or other suggestive symptoms. Women, the elderly and patients with diabetes may have "atypical" presentations.

Obtain 12 lead ECG within 10 minutes of arrival; repeat every 10 to 15 minutes if initial ECG non diagnostic but clinical suspicion remains high (initial ECG often not diagnostic).

1. STEMI: ST segment elevations $\geq$1 mm (0.1 mV) in two anatomically contiguous leads or $\geq$2 mm (0.2 mV) in leads V2 and V3, or new left bundle branch block and presentation consistent with ACS.

2. Non-STEMI or unstable angina: ST segment depressions or deep T wave inversions without Q waves or possibly no ECG changes.

Obtain emergent cardiology consultation for ACS patients with cardiogenic shock, left heart failure, or sustained ventricular tachyarrhythmia.

Initial interventions:

Assess and stabilize airway, breathing and circulation.

Attach cardiac and oxygen saturation monitors; provide supplemental oxygen as needed to maintain O2 saturation >90 percent, establish IV access.

Treat sustained ventricular arrhythmia rapidly according to ACLS protocols.

Give aspirin 325 mg (non-enteric coated), to be chewed and swallowed (unless aortic dissection is being considered). If oral administration not feasible, give as rectal suppository.

Give three sublingual nitroglycerin tablets (0.4 mg) one at a time, spaced five minutes apart, If patient has persistent chest discomfort, hypertension, or signs of heart failure and there is no sign of hemodynamic compromise (eg, right ventricular infarction) and no use of phosphodiesterase inhibitors (eg, for erectile dysfunction).

Give morphine sulfate (2 to 4 mg slow IV push every 5 to 15 minutes) for unacceptable persistent discomfort or anxiety related to myocardial ischemia.

Start 80 mg of atorvastatin as early as possible, and preferably before PCI, in patients not on statin. If patient is taking a low to moderate intensity statin, switch to atorvastatin 80 mg.

<u>Acute management STEMI:</u>

Select reperfusion strategy: Primary percutaneous coronary intervention (PCI) strongly preferred, especially for patients with cardiogenic shock, heart failure, late presentation, or contraindications to fibrinolysis. For patients with symptoms of >12 hours, fibrinolytic therapy is not indicated, but emergent PCI may be considered,

particularly for patients with evidence of ongoing ischemia or those at high risk of death.

Treat with fibrinolysis if PCI unavailable within 120 minutes of first medical contact, symptoms <12 hours and no contraindications.

Give oral antiplatelet therapy (in addition to aspirin) to all patients:

1. Patients treated with fibrinolytic therapy: Give clopidogrel loading dose 300 mg if age 75 years or less; if age over 75 years give 75 mg.

2. Patients treated with no reperfusion therapy: Give ticagrelor 180 mg.

3. Patients treated with primary percutaneous coronary intervention: Give ticagrelor loading dose of 180 mg or prasugrel loading dose of 60 mg (if no contraindications: prior stroke or TIA, or relative contraindications for prasugrel such as those age 75 years or older, weight less than 60 kg). For patients at high risk of bleeding or those for whom prasugrel or ticagrelor cannot be used, we give clopidogrel 600 mg.

Give anticoagulant therapy to all patients: enoxaparin or Unfractionated Heparin

<u>Acute management of unstable angina or non-STEMI:</u>

Give antiplatelet therapy (in addition to aspirin) to all patients:

1. Patients not treated with an invasive approach: Give ticagrelor loading dose 180 mg. For these patients who are at very high risk (eg, recurrent ischemic discomfort, dynamic ECG changes, or hemodynamic instability) consider adding a GP IIb/IIIa inhibitor (either eptifibatide or tirofiban).

2. For patients managed with an invasive approach: Give ticagrelor loading dose of 180 mg at presentation. Prasugrel loading dose of 60 mg may be used as an alternative if given after diagnostic coronary angiography.

For patients age 75 years or older, weight less than 60 kg or past stroke or transient ischemic attack, ticagrelor or clopidogrel are preferred to prasugrel. Clopidogrel may be given in a dose of 300 to 600 mg. For patients otherwise at high risk for bleeding due to prior hemorrhagic stroke, ongoing bleeding, bleeding diathesis, or clinically relevant anemia or thrombocytopenia, clopidogrel 300 to 600 mg is an option.

Give anticoagulant therapy in all patients.

Major studies with relation of Serum Bilirubin with CAD

Several studies have noted an inverse relationship between the presence of CAD and circulatory total bilirubin in the absence of liver disease.

In 1994, Schwertner et al.[121] were the first to observe Bilirubin perfusion was shown to significantly decrease infarct damage caused by Ischemic Heart Disease and Serum bilirubin concentrations in the upper range of normal values were protective against coronary artery disease and concentrations in the lower range increase atherogenic risk thus risk of Ischemic Heart Disease proven by coronary angiography.

After that study, Hopkins et al.[122] found that bilirubin was an independent protective factor with an odds ratio (OR) of 0.25 for an increase of 1 mg/dL when comparing patients with early familial CAD with control subjects. These relationships were proved in asymptomatic males who had abnormal treadmill tests and who underwent coronary angiography to rule out the presence of CAD. The reduced level of total bilirubin was correlated univariately and multivariately with the presence of

CAD and this relationship remained significant after adjustment for known CAD risk factors such as age, cholesterol, HDL cholesterol, smoking, and systolic blood pressure.[123] On the basis of these findings, one study examined the predictive ability of bilirubin for coronary artery disease which is confirmed by angiography. Bilirubin-containing ratios were found to be an independent risk predictor when tested with the traditional risk factors.[124]

Since then numerous studies have been published, which have consistently demonstrated that subjects with lower bilirubin levels are at increased risk of both coronary and peripheral atherosclerotic disease.

In addition to stenosis proved by coronary angiography, bilirubin has also been proved to be associated with coronary artery calcification (CAC) which is a good surrogate marker of the presence and amount of coronary atherosclerosis.

Tanaka et al.[125] did study consisted of 637 participants and evaluated the relationship between Coronary Artery Calcification (CAC) score determined by multislice computed tomography and serum bilirubin concentration, concluded Low serum bilirubin concentration is associated with coronary artery calcification and would be useful as a provisional new risk factor of CAC.

Also In a study by Zhang ZY et al.[40] Concluded an independent inverse association between serum total bilirubin and Coronary artery calcification scores (CAC) score in males and low serum bilirubin concentration would be useful as a potential risk factor for CAC in males.

Besides anatomic evidence of atherosclerosis, bilirubin has been investigated for association with functional markers such as coronary flow reserve (CFR)

measured by echocardiography. CFR is used to assess epicardial coronary arteries and to examine the integrity of coronary microvascular circulation.

Gullu et al.[126] investigated 160 young (18 – 45 years) healthy subjects without CAD using echocardiography. CFR values measured by stress echocardiography were significantly higher in subjects with high bilirubin concentrations than in those who were in the low bilirubin groups. This study has confirmed that serum bilirubin concentration in the upper portion of the reference interval provides protection against coronary microvascular dysfunction and CFR impairment.

Similarly Erdogan T et al.[43] found higher serum bilirubin levels with in relatively normal ranges were related with favorable coronary collateral growth in patients with Total coronary occlusion and serum bilirubin may be responsible for the difference in coronary collateral vessel development among different patients with coronary artery disease. The anti-inflammatory and anti-oxidant properties of bilirubin may mediate this effect.

Carotid atherosclerosis {e.g., intima-media thickness (IMT), plaque} is an important and sensitive surrogate marker of CVD. Study by Ryuichi Kawamoto et al.[127] showed lower serum bilirubin is strongly associated with an increased prevalence of carotid atherosclerosis in both genders and in addition to the consideration of present conventional confounding factors may be useful for individual risk assessment.

Yosuke Tatami et al.[128] found low serum bilirubin levels were associated with severe carotid atherosclerosis in CAD patients and serum bilirubin levels might be an independent, useful and cost-effective tool for evaluating atherosclerotic status in CAD patients.

Erdogan et al.[129] also found that carotid IMT was significantly greater in subjects with lower serum bilirubin concentrations. The relationship between bilirubin and carotid IMT, a marker of subclinical atherosclerosis, in nondiabetic and diabetic subjects was evaluated by Dullaart et al.[130] In this study, carotid IMT was negatively related to bilirubin after adjustment for cardiovascular risk factors in both non-diabetic and in diabetic subjects.

All consistent with data by Vítek et a.l[131] which demonstrated a marked postponement in the progression of the intimo-medial thickness of carotid arteries in hyperbilirubinemic subjects with Gilbert syndrome, when compared to normo bilirubinemic individuals.

The inverse association between serum bilirubin concentrations and CAD has been found in several studies

Troughton JA et al.[132] measured serum bilirubin in 216 participants who had developed coronary heart disease at 5-year follow-up and in 434 matched controls and found bilirubin is a novel coronary heart disease risk marker in middle-aged men, with a U-shaped relationship observed between bilirubin concentration and coronary heart disease risk.

Kaya MG et al.[133] found high Serum Total Bilirubin level is independently associated with severity of coronary artery disease in patients with NSTEMI. However, no association was found with long-term mortality.

Kim KM et al.[134] did cross-sectional study on 19,792 Koreans and found Serum total bilirubin concentration inversely correlated with Framingham risk score and it may be helpful to decrease the future risk of Coronary artery disease.

In India Veerendra Kumar Arumalla et al.[135] found plasma bilirubin concentration could act as a provisional new marker of atherogenic risk that can be measured easily in the clinical laboratory and applied in medical practice.

Also Simmi Kharb[136] found An inverse relationship between increase in total bilirubin and serum levels of LDL-C in Myocardial Infarction. Giving a possibility of bilirubin playing a role in the pathogenesis of coronary heart disease through LDL-C levels.

Zhu C et al.[137] tried to find Association of arterial stiffness (measured by brachial-ankle pulse wave velocity) with serum bilirubin levels in patients with established coronary artery disease and found level of total serum bilirubin is negatively correlated.

Song YS et al.[138] with the objective to investigate the effects of low serum bilirubin levels on the risk for future CAD in a prospective cohort of 8,593 subjects found the addition of low serum bilirubin levels to the traditional risk factors for CAD such as metabolic syndrome, may yield an improvement of risk prediction.

Dongying wang and Xue bin Cao[139] included totally 1770 non-diabetes mellitus subjects who underwent elective coronary angiography (CAG) and divided into control subgroup and coronary atherosclerotic heart disease (CAD) group in choice, patients with CAD were divided into group of angina pectoris (AP) group and that of ST-segment elevation myocardial infarction (STEMI) group and measured Serum bilirubin and lipids and concluded Serum bilirubin influence the effects of Cholesterol in the progress of CAD.

Pernette.R.W. et al.[140] Hypothesized first that high bilirubin levels might protect patients with Familial Hypercholestermia from Cardiovasular disease. Furthermore, bilirubin levels were significantly increased after treatment with simvastatin 80 mg, independent of changes in liver enzymes, which might confer additional protection against Cardiovascular disease.

The same association was also reported in a recent Taiwanese prospective study by Huang et al[141] on Patients with cardiac X syndrome followed for 5 years, in which patients with the lowest serum bilirubin levels had a higher incidence of non-fatal myocardial infarction, ischemic stroke, rehospitalization for unstable angina and coronary revascularization procedures.

Glucuronidation by UGT1A1 has a controlling effect on serum bilirubin levels.[44] Because the UGT1A1*28 Gilbert syndrome polymorphism results in higher (protective) serum bilirubin levels, it is expected to be associated with reduced CVD risk. Seven studies have addressed this, and all confirmed the association of UGT1A1*28 with higher bilirubin levels.[142]

Among them the strongest protective effect of elevated bilirubin on CVD was reported in a prospective population-based cohort stud. In the Framingham Heart Study by Jing-ping et al[42] which included 1780 unrelated individuals who had been followed up for 24 years found Homozygotes with UGT1A1*28 allele carriers with higher serum bilirubin concentrations exhibited a strong association with lower risk for Cardiovascular disease.

Some Studies tried to find Gender difference for effect of bilirubin on cardiovascular disease and found the effect of bilirubin on the risk of cardiovascular disease is apparent in men[143] but is less clear in women [41,144] Resuls of these studies

were in contarst by Djousse L et al[67] who used data collected on participants in the Framingham Offspring Study and suggested that low albumin and low bilirubin levels are associated with a greater than expected risk of myocardial infarction in men and women.

Also in the large study by Horsfall et al.[145] demonstrated the association between reduced serum bilirubin levels and increased CVD risk is strong in both sexes and could act as an independent risk factor. It seems likely that several factors are playing a role; antioxidant effects of bilirubin, heme oxygenase activity and consumption of bilirubin by oxidative processes could all be involved.

All the above studies have found inverse relationship between serum bilirubin and coronary artery disease and in future could act as risk factor for prediction.

Other beneficial effects of bilirubin

A similar inverse association has been shown between serum bilirubin concentrations and stroke.

Kimm et al.[39] found that men with higher serum bilirubin levels had a lower hazard ratio for ischaemic stroke after adjustment for multiple confounding risk factors compared with men in the lowest bilirubin quartile (OR 0.66; 95% confidence interval (CI) 0.49 – 0.89, P 0.0016).

Perlstein et al.[146] had similar findings in a cross-sectional cohort study that consisted of 7075 adults enrolled in the National Health and Nutrition Examination Survey (NHANES) and included both sexes and various racial groups. In their study, they found that each 0.1 mg/dL increase in serum bilirubin led to a 9% reduced odds of stroke.

Perlstein et al.[147] also reported inverse association of bilirubin with peripheral artery disease (PAD) in the NHANES (1999 to 2004). In that study a 0.1 mg/dL increase in bilirubin level was associated with a 6% reduction in the odds of PAD after adjustment for cardiovascular risk factors. The inverse association of bilirubin with PAD tended to be stronger among men compared with women.

There are also numerous studies indicating serum bilirubin levels being associated with Metabolic syndrome or Diabetes. The first report was about the association of bilirubin with metabolic syndrome among children and adolescents.[148]

From NHANES between 1999 and 2006, 15,876 participants were selected for study. After age adjustment, increased total bilirubin was associated with a 26%

reduction in diabetes risk. Multivariate analysis, adjusting for all diabetes risk factors assessed, confirmed this association.[149]

The study of a very large number of subjects in the United States revealed that the odds ratio for the history of colorectal cancer is reduced to 0.295 in men and 0.186 in women per 1 mg/dL increment in serum bilirubin levels.[150]

Some of the studies did not find any relation to genetically raised serum bilirubin

A study of 43,708 people in Denmark demonstrated that the inverse association of total bilirubin with ischemic heart disease and myocardial infarction becomes not significant after further adjustment for more cardiovascular risk factors.[150] Meta-analysis of studies involving 14,711 case and 60,324 controls also has not revealed a causal relationship between total bilirubin and cardiovascular diseases.[151]

Eight studies reported no significant association between genetically elevated bilirubin and risk of Myocardial infarction[152-154], peripheral arterial disease[155], ischaemic stroke[156] or coronary artery disease[157,158] or severity of coronary artery disease[159]

It has been suggested that the inconsistent results could be owing to differences in study designs and/or characteristics of participants and a possible explanation for this is that serum bilirubin is determined not only by excretion, but is also a reflection of its production rate. In this aspect, increased activity of HO, may provide an explanation for the observed inverse association between serum bilirubin and CAD because of the cardioprotective effects of increased elimination of heme or enhanced production of CO an active vasodilator. Another potential limitation is that

life long genetically elevated bilirubin might cause a downregulation of other endogenous antioxidants not measured in these studies, in effect neutralizing the putatively protective effects of raised bilirubin levels.

Some studies found raised levels

In the Simvastatin and Ezetimibe in Aortic Stenosis study[160], a randomized trial studying the effects of simvastatin and ezetimibe therapy on cardiovascular adverse events in 1,873 patients with mild-to-moderate, asymptomatic aortic stenosis, higher total bilirubin levels were associated with higher total mortality risk. In another study of patients with ST-elevation myocardial infarction (STEMI) high total bilirubin levels were associated with higher risk of cardiovascular mortality, advanced heart failure, and adverse cardiac events after primary coronary intervention.[161] In fact, total bilirubin levels have been reported to be elevated, probably due to an increase in stress-induced HO-1 activity after acute myocardial infarction[162] and total bilirubin levels are positively associated with the severity of coronary artery disease in patients with both STEMI[163] and non-STEMI[133]. Therefore, the association of high total bilirubin levels with higher total mortality risk could be a reflection of stress-induced activation of HO-1 increasing total bilirubin levels as a protective mechanism.

Hence Aim of my study is to find relation between Serum Bilirubin and Acute Coronary Syndrome.

MATERIALS AND METHODS

SOURCE OF DATA:

The patients attending the medicine OPD and admitted to B.L.D.E.U's I.C.U Shri.B. M. Patil Medical College, Hospital and Research Centre, Vijayapur who fulfill the inclusion criteria from October 2013 to April 2015.

SAMPLE SIZE:

The prevalence of coronary artery disease in India is 8%[25]. At 95% confidence interval and margin error $\pm$ 5, the sample size is 113 using the statistical formula

$$n = \frac{(1.96)^2 \times p \times q}{d^2}$$

p= prevalence of Acute Coronary Syndromes

q= (100 – p)

d= Margin error $\pm$ 5

STATISTICAL ANALYSIS:

Data will be presented diagrammatically and by Mean $\pm$SD

Correlation coefficient

METHOD OF COLLECTION OF DATA

STUDY DESIGN

A descriptive cross sectional study design will be used.

METHOD OF COLLECTION OF DATA

A detailed history, general physical examination, systemic Examination and investigations will be performed on all patients who fulfill the inclusion criterion and age >18yrs, both sexes who are admitted in ICU in BLDEU'S Shri B.M PATIL Medical college hospital and research centre Vijayapur.

INCLUSION CRITERIA:

1. The non-ST segment elevation MI, ST segment elevation MI and Unstable anginas are selected on basis of history, examination and relevant investigations.

EXCLUSION CRITERIA:

1. Hepatitis of any cause.
2. On drugs like Vitamin C, Amiodarone

   Oral Hypoglycemics: Acarbose, Pioglitazone,

   Anti-seizure drugs: Carbamazepine, Felbamate, ValproicAcid, and Phenobarbitol

   Anti fungal Drugs: Itraconazole, Ketoconazole and Terbinafine

   Anti Tuberculosis: Isoniazid, Pyrazinamide, and Rifampicin

   Antiretroviral Drugs: Ritonavir, Nevirapine

3. Non cardiac chest Pain.
4. Cirrhosis of Liver
5. Bile duct obstruction
6. Recent (less than 3 months old)

    Major Trauma

    Surgery

    Burns
7. Myocardial Re-Infarction patient
8. Haemolytic jaundice

LABORATORY

PRINCIPLE:

Sulfanilic acid reacts with sodium nitrite to form diazotized sulfanilic acid. Total Bilirubin reacts with diazotized sulfanilic acid in the presence of DMSO (Di Methyl Sulf Oxide) to form azobilirubin

NORMAL RANGE:

0.2 to 1.0 mg/dl, Mean 0.6 mg/dl

SAMPLE:

Serum/plasma (free of haemolysis)

PROCEDURE:

Total bilirubin reagent-1000 μL

Serum-50μL

Mix well and incubate for 5 minutes exactly. Measure the absorbance of the sample against the respective sample blank at 546 or 532nm

CALCULATION:

For semi auto with factor:

Total bilirubin = OD of test – OD of sample blank × Factor

With artificial standard:

$$\text{Total bilirubun concentration} = \frac{\text{OD of test} - \text{OD of sample blank}}{\text{OD of standard}}$$

RESULTS

1) Gender

In the 113 patients with Acute Coronary Syndrome (ACS) studied 72.6% were male and 27.4 % females.

Table 2-Demonstrating gender distribution.

Gender	Frequency	Percentage
Male	82	72.6
Female	31	27.4
Total	113	100

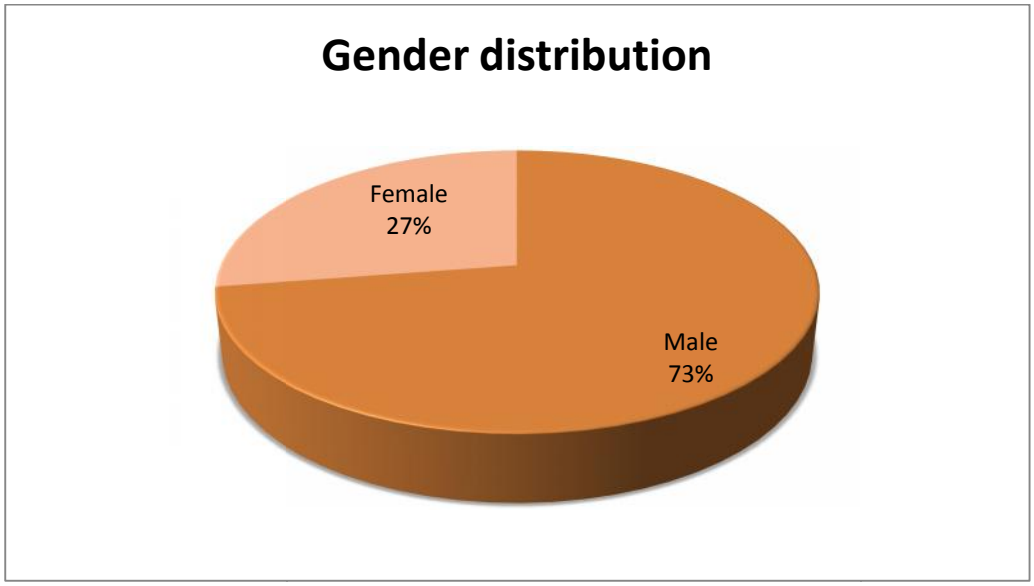

Figure 11-Demonstrating gender distribution

2) Age

The age ranged from 26 to 95 years of age. The mean age of the group was 54.5 $\pm$ 13.19 SD. The patients in the age group of 55 to 65 years had the highest incidence of ACS.

Table 3-Demonstrating age distribution

Age (years)	Frequency	Percentage
25-35	7	6.2
35-45	12	10.6
45-55	19	16.8
55-65	36	31.9
65-75	30	26.5
>=75	9	8.0
Total	113	100.0

Mean (SD) = 54.5 (13.19)

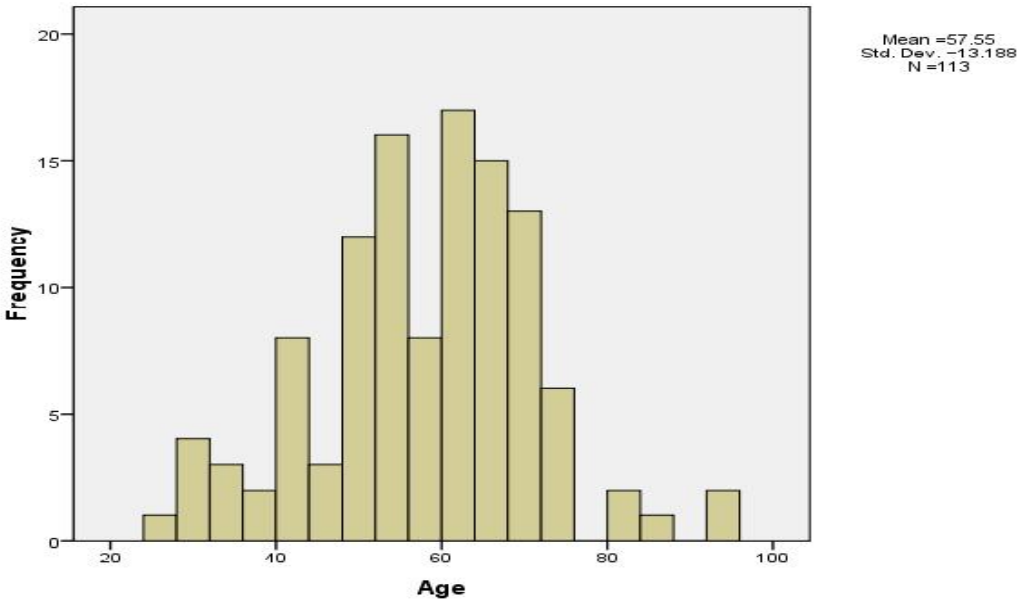

Figure 12-Demonstrating age distribution

3) Risk Factors

The known risk factors of ACS like smoking status, tobacco chewing, diabetes mellitus, family history of ACS and hypertension were studied and correlated. 37.2 % of all ACS patients were smokers, 31% chewed tobacco, 24.7 % had diabetes mellitus, 8 % had family history of ACS and 31.8% were hypertensive.

Hypertension had statistically significant correlation with ACS. All risk factors were more associated with STEMI compared to unstable angina or NSTEMI.

Table 4-Relation of risk factors with components of ACS

	ACS					
Parameters	Unstable	NSTEMI	STEMI	Total	χ^2 value	p-value
Smoking	3	3	36	42	5.80	0.02
Tobacco chewer	5	9	21	35	4.18	0.12
Diabetes status	3	7	18	28	1.32	0.52
Family History	1	3	5	9	1.42	0.23
HTN	8	12	16	36	22.62	<0.0001

4) Correlation of LDL and Total leucocyte count with bilirubin.

Total Leucocyte count (TC) in ACS is usually high as infarction of the myocardium is an inflammatory process. In our study the mean TC was 13785.7 ± 5140.27 SD. The minimum TC was 2000 cells/cumm and maximum was 30730 cells/cumm.

Mean LDL was 172.07 ± 46.57 which was higher than the normal considering the cut off value with existing risk factors. The minimum LDL value was 70 mg/dl whereas the maximum was 291.8mg/dl

Both TC and LDL were statistically significant when compared to bilirubin levels

Table 5-Distribution of LDL, TC and Total Bilirubin.

Variable	Minimum	Maximum	Mean	SD
LDL	70	291.8	172.07	46.57
TC	2000	30730	13785.7	5140.27
Total Bilirubin	0.1	2.1	0.48	0.28

Table 6-Correlation between Total Bilirubin with LDL ad TC

	Pearson's correlation coefficient value (r)	p-value
LDL	-0.755	<0.0001
TC	-0.787	<0.0001

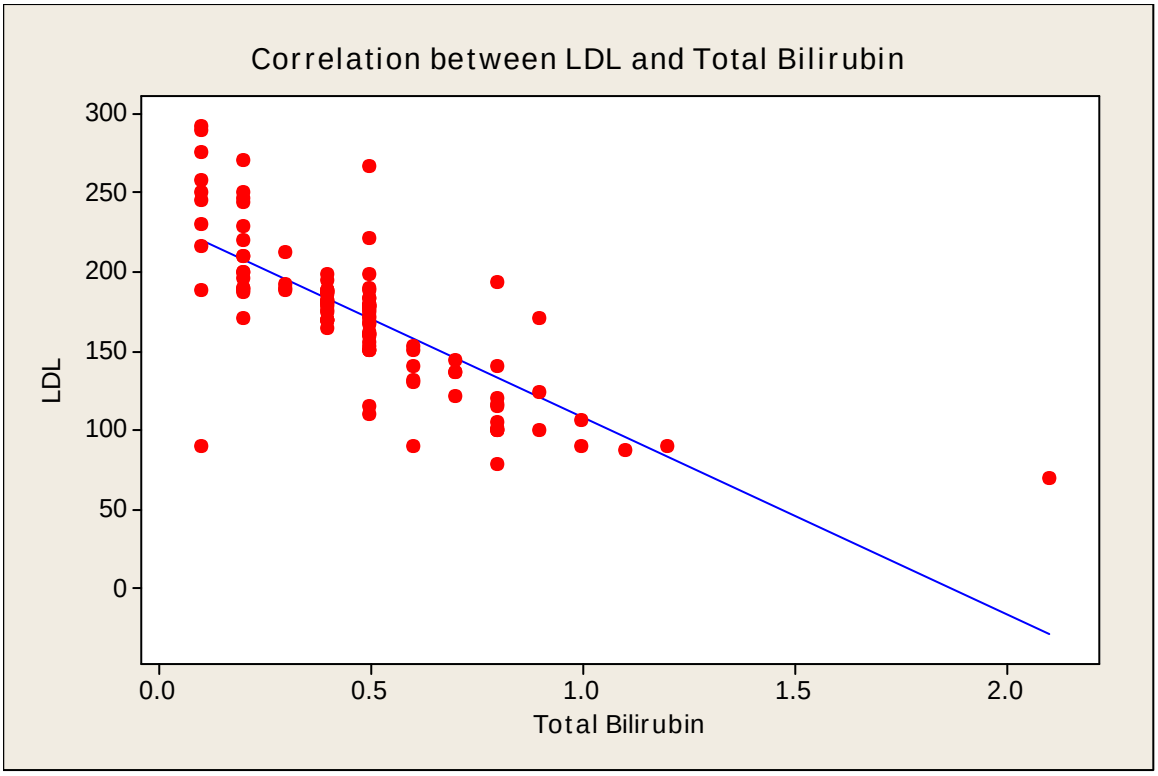

Figure 13-Correlation between Total Bilirubin and LDL

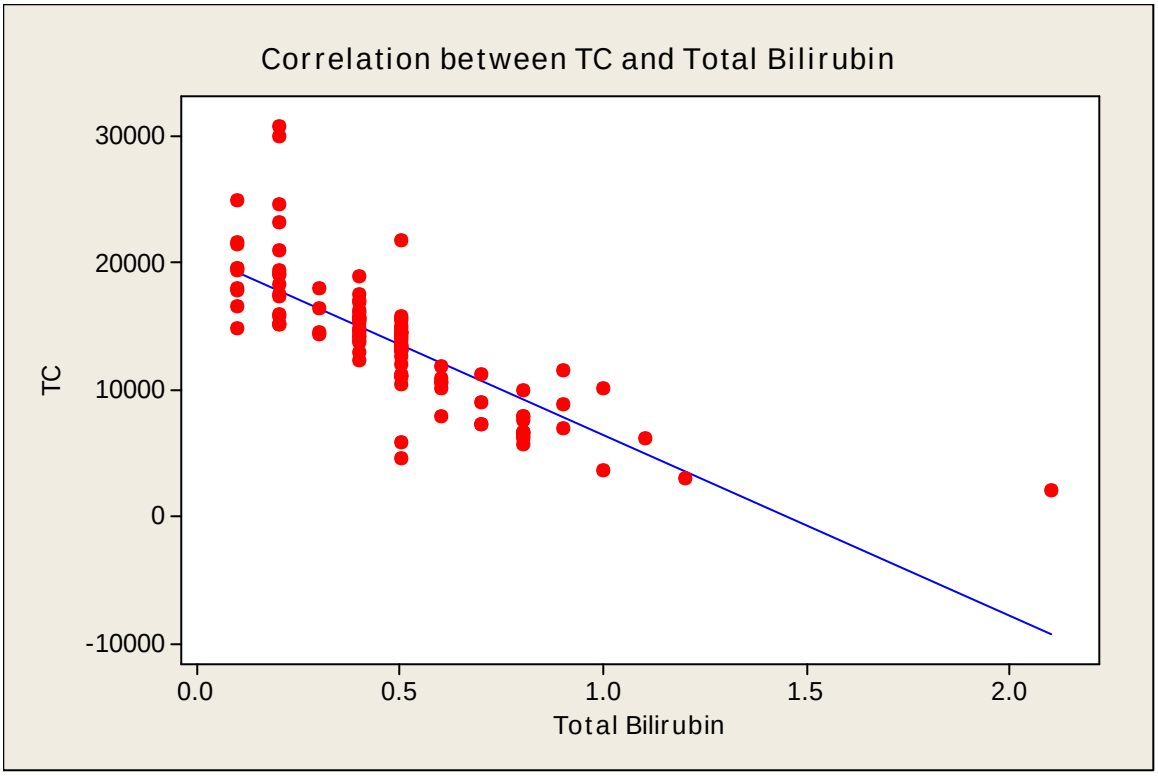

Figure 14-Correlation between Total Bilirubin and TC

Relation of Total Bilirubin with ACS

Table 7-Distribution of Total Bilirubin with components of ACS

Total Bilirubin	N	Mean	Std. Deviation	Minimum	Maximum
STEMI	82	.484146	.3069039	.1000	2.1000
NSTEMI	21	.452381	.1691717	.2000	.8000
Unstable	10	.530000	.2869379	.1000	1.0000
Total	113	.482301	.2832308	.1000	2.1000

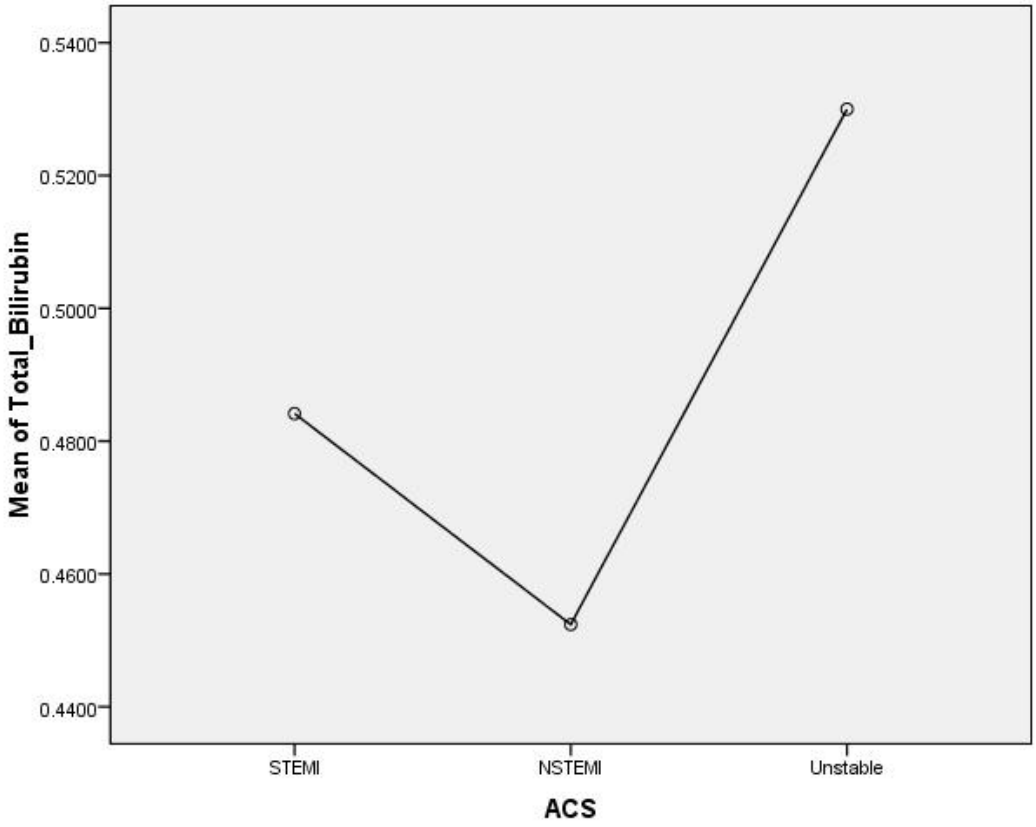

Figure 15-Distribution of Total Bilirubin with components of ACS

One-sample comparison with standard normal mean bilirubin value = 0.6

Table 8-Comparision of Total Bilirubin in patients with ACS and standard normal mean bilirubin

	ACS	Mean difference	95% CI of difference	t-value	p-value
Total Bilirubin	0.48 $\pm$ 28	0.12	0.06 – 0.17	4.42	P<0.0001

There is statistically significance, less total bilirubin of ACS than standard normal mean bilirubin value (0.6) ($p<0.0001$).

DISCUSSION

This study was conducted on 113 patients admitted with acute coronary syndrome at ShriB.M.Patil medical college, hospital and research centre from October 2013 to April 2015. Of the study group 72.6% were male and 27.4% were female. The male preponderance was similar to other studies like Sahen O et al[133] were 66% of participants were male and 34% were female. Hopkins et al[122] had 75.6% males and 24.4% females.

Table 9-Distribution of sex with current and other studies

Study	Male (%)	Female (%)
Sahen O et al[133]	66	34
Hopkins et al[122]	75.6	24.4
Present study	**72.6**	**27.4**

The age ranged from 26 to 95 years of age. The mean age of the group was 54.5 $\pm$ 13.19 SD. The patients in the age group of 55 to 65 years had the highest incidence of ACS. This finding was similar to other studies and accepted fact that the incidence of ACS increases with age.

The known risk factors of ACS like smoking status, tobacco chewing, diabetes mellitus, family history of ACS and hypertension were studied and correlated. 37.2 % of all ACS patients were smokers, 31% chewed tobacco, 24.7 % had diabetes mellitus,8 % had family history of ACS and 31.8% were hypertensive.

Hypertension had statistically significant correlation with ACS. All risk factors were more associated with STEMI compared to unstable angina or NSTEMI. On comparing with similar studies all risk factors had positive correlation with ACS.

In a study done by Hopkins P N et al[122] 52.2 % were smokers, 47.2 % had hypertension and 14.3 % had diabetes mellitus.

Sahin O et al[133] in their study had 36% smokers,50 % were hypertensive and 26 % were diabetics.

Table 10-Distribution of risk factors with current and other studies

Study	Smokers (%)	Hypertensive (%)	Diabetes mellitus(%)
Sahin O et al[133]	36	50	26
Hopkins P N et al[122]	52.2	47.2	14.3
Present study	**37.2**	**31.8**	**24.7**

Total Leucocyte count (TLC) in ACS is usually high as infarction of the myocardium is an inflammatory process. In our study the mean TLC was 13785.7 $\pm$ 5140.27 SD.

Mean LDL was 172.07 $\pm$ 46.57 which was higher than the normal considering the cut off value with existing risk factors.

Both TLC and LDL were statistically significant when compared to bilirubin levels and hence our study was similar to other studies in this regard.

Bilirubin has been long postulated to have antioxidant properties and thus its correlation with ACS is of interest. This study tried to find association of serum total bilirubin levels in patients with acute coronary syndrome.

The mean bilirubin values in mg/dl for STEMI was 0.48 ± 0.30 SD ,for NSTEMI it was 0.45 ± 0.16 and for unstable angina it was 0.28 ± 0.1 SD.

The mean total bilirubin in patients with ACS was 0.48 ± 0.28 SD mg/dl compared to bilirubin levels of 0.6mg/dl in normal population.

There is statistically significance less total bilirubin of ACS than normal standard mean value (0.6) ($p<0.0001$). Hence our research hypothesis accepted.

The results were comparable to Sahin O et al[133] and Hopkins P N et al[122] who also had decreased bilirubin levels in patients with ACS.

Table 11-Distribution of Total Bilirubin with current and other studies

Study	Total Bilirubin levels
Sahen O et al[133]	0.59 ± 0.17
Hopkins P N et al[122]	0.52 ± 6.1
Present Study	**0.48 ± 0.28**

Substantial evidence has documented that the development of CAD involves lipid oxidation and formation of oxygen radicals as atherosclerosis and inflammation are associated with formation of oxygen and peroxyl radicals.[12-17,19,22-26,37-39,41-58]

Bilirubin has proven to be a potent antioxidant under physiological conditions by inhibiting both lipid and protein oxidation.[20] In several studies it was found that different circulating forms of bilirubin are powerful antioxidants: Free bilirubin, albumin-bound bilirubin, conjugated bilirubin, and unconjugated bilirubin were all noted to be effective scavengers of peroxyl radicals and to be able to protect human

LDL against peroxidation[24].Additionally, bilirubin exerts anti-inflammatory effects on vasculature and inhibits proliferation of vascular smooth muscle cells.[22,23]

This has led to suggestions that mildly increased circulatory bilirubin may have a physiologic function to protect against disease processes that involve oxygen and peroxyl radicals or vice versa and many studies have shown relation with CAD.[39-43]

Reduced levels of bilirubin were shown to be associated with higher prevalence of coronary artery disease emerging as new potential risk factor marker.[25]

The inverse association between serum bilirubin concentrations and CAD has been found in several studies

Troughton JA et al.[132] found bilirubin is a novel coronary heart disease risk marker in middle-aged men, with a U-shaped relationship observed between bilirubin concentration and coronary heart disease risk.

Sahin O et al.[133] found high serum total bilirubin level is independently associated with severity of coronary artery disease in patients with NSTEMI.

Kim KM et al.[134] did cross-sectional study on 19,792 Koreans and found Serum total bilirubin concentration inversely correlated with Framingham risk score and it may be helpful to decrease the future risk of Coronary artery disease.

In India, Veerendra Kumar Arumalla et al.[135] found plasma bilirubin concentration could act as a provisional new marker of atherogenic risk that can be measured easily in the clinical laboratory and applied in medical practice.

Also Simmi Kharb found an inverse relationship between increase in total bilirubin and serum levels of LDL-C in Myocardial Infarction. Giving a possibility of bilirubin playing a role in the pathogenesis of coronary heart disease through LDL-C levels.[136]

Song YS et al.[138] with the objective to investigate the effects of low serum bilirubin levels on the risk for future coronary artery disease (CAD) in a prospective cohort of 8,593 subjects found the addition of low serum bilirubin levels to the traditional risk factors for CAD, such as metabolic syndrome, may yield an improvement of risk prediction.

The same association was also reported in a recent Taiwanese prospective study by Huang et al[141] on Patients with cardiac X syndrome followed for 5 years, in which patients with the lowest serum bilirubin levels had a higher incidence of non-fatal myocardial infarction, ischemic stroke, rehospitalization for unstable angina and coronary revascularization procedures.

Glucuronidation by UGT1A1 has a controlling effect on serum bilirubin levels.[44] Because the UGT1A1*28 Gilbert syndrome polymorphism results in higher (protective) serum bilirubin levels, it is expected to be associated with reduced CVD risk. Seven studies have addressed this, and all confirmed the association of UGT1A1*28 with higher bilirubin levels.[142]

Among them the strongest protective effect of elevated bilirubin on CVD was reported in a prospective population-based cohort study In the Framingham Heart Study by Jing-ping et al[42] which included 1780 unrelated individuals who had been followed up for 24 years found Homozygotes with UGT1A1*28 allele carriers with

higher serum bilirubin concentrations exhibited a strong association with lower risk for Cardiovascular disease.

Also in the large study by Horsfall et al[145] demonstrated the association between reduced serum bilirubin levels and increased CVD risk is strong in both sexes and could act as an independent risk factor. It seems likely that several factors are playing a role; antioxidant effects of bilirubin, heme oxygenase activity and consumption of bilirubin by oxidative processes could all be involved.

CONCLUSION

Our study showed an inverse correlation of bilirubin with ACS. This reinforces the fact that bilirubin acts as an antioxidant and has cardioprotective action and patients with ACS have lower levels of bilirubin. This can used as a factor for screening individuals who have high risk for ACS and preventive strategies applied in them before the onset of overt ACS.

SUMMARY

Study group comprised 113 cases of ACS admitted in B.L.D.E.U's I.C.U Shri B.M. Patil Medical College, Hospital and Research Centre, Vijayapur based on the inclusion and exlusion criterias between DECEMBER 2013 to APRIL2015.

1) Of the 113 cases, 72.6% were male and 27.4 % females.

2) The age ranged from 26 to 95 years of age. The mean age of the group was 54.5 $\pm$13.19 SD. The patients in the age group of 55 to 65 years had the highest incidence of ACS.

3) The known risk factors of ACS were studied and correlated. 37.2 % of all ACS patients were smokers 31% chewed tobacco, 24.7 % had Diabetes Mellitus,8 % had family history of ACS and 31.8% were hypertensive.

Hypertension had statistically significant correlation with ACS. All risk factors were more associated with STEMI compared to unstable angina or NSTEMI.

4) On Correlation of LDL and Total leucocyte count with bilirubin both were statistically significant when compared to bilirubin levels.

5) The mean bilirubin values in mg/dl for STEMI was 0.48 $\pm$ 0.30 SD ,for NSTEMI it was 0.45 $\pm$ 0.16 and for unstable angina it was 0.28 $\pm$ 0.1 SD. The mean total bilirubin in patients with ACS was 0.48 $\pm$ 0.28 SD mg/dl compared to bilirubin levels of 0.6mg/dl in normal population. There is statistically significance, less total billirubin of ACS than normal standard mean value (0.6) ($p<0.0001$)

6) This study showed inverse inverse correlation of bilirubin with ACS and can be used as a factor for screening individuals who have high risk for ACS and preventive strategies applied in them before the onset of overt ACS.

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ETHICAL CLERANCE CERTIFICATE

No. 50/13
Date 14-11-13
BLDE U's Shri B.M. Patil Medical College

B.L.D.E.UNIVERSITY'S
SHRI.B.M.PATIL MEDICAL COLLEGE, BIJAPUR-586 103
INSTITUTIONAL ETHICAL COMMITTEE

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Ethical Committee of this college met on 13-11-2013 at 3-30pm to scrutinize the Synopsis of Postgraduate Students of this college from Ethical Clearance point of view. After scrutiny the following original/corrected & revised version synopsis of the Thesis has been accorded Ethical Clearance.

Title "Study of total serum bilirubin in acute coronary syndrome"

— x — x — x —

Name of P.G. student Dr. Gudage Nitin
Department of Medicine

Name of Guide/Co-investigator Dr Anand. P. Ambali.
Professor of Medicine

DR.TEJASWINI. VALLABHA
CHAIRMAN
INSTITUTIONAL ETHICAL COMMITTEE
BLDEU'S, SHRI.B.M.PATIL
MEDICAL COLLEGE, BIJAPUR.

Following documents were placed before E.C. for Scrutinization
1) Copy of Synopsis/Research project.
2) Copy of informed consent form
3) Any other relevant documents.

CONSENT FORM

TITLE OF RESEARCH: "STUDY OF TOTAL SERUM BILIRUBIN IN ACUTE CORONARY SYNDROME."

GUIDE : DR. ANAND.P.AMBALI

P.G. STUDENT : DR. GUDAGE NITIN

PURPOSE OF RESEARCH:

I have been informed that the purpose of this study is to find out whether there is any association between acute coronary syndrome and total bilirubin.

PROCEDURE:

I understand that I will undergo detailed history and clinical examination and investigations.

RISKS AND DISCOMFORTS:

I understand that there is no risk involved and I may experience mild pain during the above mentioned procedures.

BENEFITS:

I understand that my participation in this study will help to find out whether there is any association between acute coronary syndrome and total bilirubin.

CONFIDENTIALITY:

I understand that the medical information produced by the study will become a part of hospital record and will be subjected to confidentiality and privacy regulations

of hospital. If the data is used for publications the identity of the patient will not be revealed.

REQUEST FOR MORE INFORMATION:

I understand that I may ask for more information about the study at any time.

REFUSAL OR WITHDRAWAL OF PARTICIPATION:

I understand that my participation is voluntary and I may refuse to participate or withdraw from study at any time.

INJURY STATEMENT:

I understand in the unlikely event of injury to me during the study I will get medical treatment but no further compensations.

(Signature of Guardian) (Signature of patient)

Contact no-

(If the patient is conscious, well oriented and fully aware)

BLDEU'S SHRI B.M.PATIL MEDICAL COLLEGE

HOSPITAL AND RESEARCH CENTRE, BIJAPUR

"STUDY OF TOTAL SERUM BILIRUBIN IN ACUTE CORONARY SYNDROME"

PROFORMA

Name: **IP. No:**

Age: **Address**

Sex: **Date of Admission:**

Occupation: **Unit:**

Religion:

Contact no :

Chief complaints: Chest pain

Site/Location

Type/Character

Radiation

Vomiting

Sweating

Present history:

Past history:

History of (H/o) hypertension

H/o myocardial infarction / Angina

H/o diabetes mellitus

H/o hepatitis

H/o drug intake:

Drugs		YES/NO
1)ORAL HYPOGLYCEMICS	Acarbose,Rosiglitazone, Pioglitazone	
2)ANTI-SEIZURE	Carbamazepine, Felbamate, Valproic Acid, Phenobarbitone	
3)ANTI-FUNGAL	Itraconazole, Ketoconazole, and Terbinafine	
4)ANTI-TUBERCULAR	Isoniazid, Pyrazinamide, and Rifampin	
5)ANTI-RETROVIRAL	Ritonavir, Nevirapine	
OTHERS	1)Acetaminophen 2)Amiodarone	

H/o a) Recent Trauma, Surgery, Burns

Personal history:

Diet:

Appetite:

Sleep:

Bladder and bowel habits:

Habits:

Family history:

GENERAL PHYSICAL EXAMINATION

Pallor:

Icterus:

Cyanosis:

Clubbing:

Lymphadenopathy:

Edema:

JVP:

VITAL SIGNS:

Pulse rate:

Blood pressure:

Temperature:

Respiration rate:

SYSTEMIC EXAMINATION

CARDIOVASCULAR SYSTEM:

RESPIRATORY SYSTEM:

PER ABDOMEN EXAMINATION:

CENTRAL NERVOUS SYSTEM:

INVESTIGATIONS:

HAEMATOLOGY –

PATHOLOGY

1)Complete blood count:	
Hb	gm/dl
Total count	Cells/cumm
Differential count	
Neutrophils	%
Lymphocytes	%
Eosinophils	%
Basophils	%
Monocytes	%
Platelet Count	Cells/cumm
ESR	At end of 1st hour.
2)Urine complete	
Urine pH	
Urine color	
Urine Bile salt and pigment	
Urine albumin	
Urine sugar	
Urine microscopy RBC's Pus cells Cast's Epithelial cells	

BIOCHEMISTRY

1)Random Blood sugar	
2) Serum creatinine	
3)FASTING LIPID PROFILE	
Total Cholesterol	
Triglycerides	
HDL-Cholesterol	
LDL-Cholesterol	
VLDL-Cholesterol	
4) CPK-MB/ TROP T	
5)LIVER FUNCTION TESTS	
Total Bilirubin	

ECG-

2D-ECHO

Diagnosis

KEY TO MASTER CHART

For Sex		
Male	M	
Female	F	
For		
Smoking status	YES-1	NO-2
Tobacco chewer	YES-1	NO-2
Diabetes status	YES-1	NO-2
Family history	YES-1	NO-2
Hypertension (HTN)	YES-1	NO-2
For		
STEMI	a	
NSTEMI	b	
UA	c	

MASTER CHART

SL	Name	IP no	Age(yrs)	Sex	Smoking status	Tobacco chewer	Diabetes status	Family History	HTN	LDL	TC	Total Bilirubin	NSTEMI	Unstable Angina	STEMI
1	Arvind	1516	40	M	2	2	2	2	2	170.4	17,570	0.2			a
2	Mohammad Ismillah	1362	57	M	2	1	2	2	2	151.1	12,670	0.5			a
3	Mahadev	1693	50	M	2	2	2	2	2	136	9,000	0.7			a
4	Shivaji	1588	50	M	2	1	2	2	2	87	6,170	1.1			a
5	Gangawwa	1784	60	F	2	1	2	2	2	164.9	12,300	0.4			a
6	Shivavva	1739	42	F	2	2	1	2	2	150	10,410	0.5			a
7	Suresh	9180	30	M	1	2	2	1	2	210	15,190	0.2			a
8	Somshekar	8968	70	M	2	2	2	2	1	245	17,800	0.1			a
9	Sitabaai	8038	50	F	2	2	1	2	2	187	30,730	0.2			a
10	Savitri	9839	65	F	2	2	2	2	2	190	15,100	0.2			a
11	Prakash	10915	26	M	1	2	2	2	2	70	2,000	2.1			a
12	Abdul Saab	10799	57	M	2	1	1	2	2	100	8,770	0.9			a
13	Govind	10696	62	M	2	2	1	1	1	90	10,000	1		c	
14	Arjun	10907	66	M	1	2	2	2	2	170	18,900	0.4			a
15	Lalbee	10383	40	F	2	2	1	2	1	190	14,500	0.3			a
16	Borawwa	10939	65	F	2	1	2	2	2	210	24,600	0.2			a
17	Kallappa	11100	55	M	2	1	1	2	1	160	13,450	0.5		c	
18	Shankargouda	12409	28	M	1	1	2	2	2	220	23,220	0.2			a
19	Sadashiv	12263	58	M	1	2	2	2	1	178	13,806	0.4			a
20	Shivanand	18813	48	M	2	1	2	2	2	190	15,720	0.2	b		
21	Lakshmann	18900	55	M	1	2	2	2	2	176	13,430	0.5			a
22	Malasidda	18904	40	M	2	1	2	2	2	186	16,000	0.4	b		
23	Ningappa	18825	39	M	1	2	2	2	1	230	18,000	0.1		c	
24	Lakshmibai	18413	70	F	2	1	2	2	2	140	9,870	0.8		c	
25	Kaaveri	18728	70	F	2	2	2	2	1	120	6,540	0.8		c	
26	Keerappa	19319	70	M	1	1	2	2	2	100	5,608	0.8	b		
27	Hanamanthraya	20576	55	M	1	1	2	2	2	115	4,570	0.5			a
28	Amrutamma	7613	60	F	2	2	1	2	1	200	18,280	0.2	b		
29	Sangabasayya	20971	70	M	1	2	2	2	2	160	15,765	0.5	b		
30	Kalappa	20516	70	M	1	2	2	2	2	170	13,700	0.4			a
31	Erappa	20283	62	M	1	2	2	2	1	90	10,100	0.6	b		
32	Kasturi	21755	54	F	2	2	1	2	2	150	11,000	0.5			a
33	Sadashiv	22116	50	M	1	2	2	2	2	212	16,450	0.3			a
34	Akawwa	22499	70	F	2	2	2	2	2	200	19,000	0.2			a
35	Kamalabaai	23755	60	F	2	2	1	2	2	100	6,300	0.8			a
36	Basapaa	9048	75	M	1	2	2	2	2	110	5,764	0.5			a
37	Darappa	23739	70	M	2	1	2	2	2	150	10,890	0.6			a

38	Mallangouda	23737	52	M	1	2	1	2	2	155	11,940	0.5			a
39	Prakash	8090	55	M	2	1	2	2	2	153	11,110	0.5			a
40	Mallikarjun	23453	75	M	2	1	2	2	1	130	7,900	0.6			a
41	Boramma	23474	55	F	2	1	2	2	2	188	14,400	0.3			a
42	Timanna	23356	52	M	1	2	1	2	2	290	19,600	0.1		c	
43	Gabeesaab	22381	95	M	2	1	2	2	2	170	12,910	0.4			a
44	Moulaasaab	23635	60	M	1	2	2	2	2	144	7,300	0.7			a
45	Shivappa	23842	66	M	1	2	2	2	1	137	7,300	0.7			a
46	Apanna	38597	62	M	2	2	1	2	2	159	10,980	0.5			a
47	Monappa	20382	45	M	1	2	2	2	2	182	14,250	0.4			a
48	Durgappa	20688	55	M	1	2	2	2	2	124	6,900	0.9			a
49	Manohar	31138	42	M	1	1	2	2	2	78.4	7,930	0.8			a
50	Basappa	37958	70	M	2	2	2	2	1	267	21,820	0.5	b		
51	Revatti Mallikarjun	23593	56	F	2	2	1	1	2	175	13,550	0.5	b		
52	Hanamanth	25582	40	M	2	2	2	1	2	291.8	21,380	0.1			a
53	Annaraya	29662	52	M	2	2	2	2	2	186.6	14,800	0.4			a
54	Veerupakshappa	33634	39	M	2	1	2	2	2	250	15,970	0.2			a
55	Mahesh B	33447	31	M	1	2	2	2	2	243.8	19,100	0.2			a
56	Nagappa B	33892	60	M	1	1	2	2	2	250	19,550	0.1			a
57	Mallikarjun	37485	50	M	1	2	2	1	2	258	14,740	0.1			a
58	Hanumanth	37520	61	M	1	2	2	2	1	175	15,730	0.4			a
59	Jaibhim	32346	48	M	2	2	2	2	2	170.2	11,500	0.9			a
60	Dattatray Inamdar	32253	60	M	1	2	2	2	2	270	17,300	0.2			a
61	Vittal	33466	54	M	2	1	1	2	2	176.2	14,640	0.4			a
62	Gundappa	37049	62	M	2	2	2	2	1	189	21,009	0.2	b		
63	Basappa	36804	65	M	2	2	2	2	2	181.4	14,170	0.4			a
64	Sanju	36841	31	M	2	2	2	2	2	180.6	15,430	0.4			a
65	Yellawwa	33725	50	M	1	2	2	2	2	247	29,920	0.2			a
66	Sharnappa	33636	45	M	1	2	2	2	2	90.2	2,970	1.2			a
67	Annapurna	30582	55	F	2	2	2	2	2	106.8	3,600	1			a
68	Mallappa	29928	42	M	1	2	1	2	2	195.2	16,900	0.4			a
69	Kamalabai	30566	85	F	2	2	1	2	1	193.2	6,100	0.8			a
70	Lkashmibaai	35755	75	F	2	2	2	2	1	216.6	16,490	0.1			a
71	Ramachandra	36025	44	M	2	2	2	2	2	117	6,540	0.8			a
72	Geetabaai	28889	82	F	2	2	2	2	2	100.6	7,600	0.8			a
73	Shivalingapa	29005	75	M	2	2	2	2	2	104.4	7,880	0.8			a
74	Gauramma	29128	50	F	2	2	2	2	1	170.6	13,000	0.5			a
75	Arjun	28271	56	M	2	2	1	2	2	180	13,900	0.5			a
76	Borawwa	28749	70	F	2	2	2	2	2	178.2	14,800	0.5			a
77	Chandran	28023	60	M	1	2	2	2	2	121.6	11,140	0.7			a
78	G.Veeresh	25746	32	M	2	2	2	2	1	276.2	24,860	0.1			a
79	Balwanthappa	28360	55	M	1	2	2	2	2	192	17,950	0.3			a
80	Suresh	24411	35	M	1	2	2	2	2	189	21,600	0.1			a
81	Baburlaal	24179	65	M	2	2	2	2	2	178	11,050	0.5			a

82	Shakuntala	24355	80	F	2	2	2	2	2	183.6	13,000	0.5	b		
83	Bhimanabaai	25717	65	F	2	2	1	2	2	172.6	14,490	0.5			a
84	Basvaaraj	24119	34	M	2	2	2	2	1	180	14,530	0.5			a
85	Eranna	29759	50	M	2	2	1	2	2	140.6	10,600	0.6			a
86	Rukmuddin	36817	65	M	1	2	2	2	2	131.4	10,530	0.6			a
87	Jalilsaab	36694	72	M	2	2	1	2	1	196	19,470	0.2	b		
88	Maleshappa	24026	93	M	2	1	2	2	1	100	6,420	0.8			a
89	Kamala	24035	61	F	2	2	1	2	2	90	19,400	0.1			a
90	Vijay	24127	55	M	2	1	2	2	1	115	6,600	0.8	b		
91	Abbasab	24022	56	M	2	2	2	2	1	229	15,160	0.2			a
92	Basanna	19136	60	M	2	2	2	2	1	187	15,620	0.4			a
93	Shivappa	19294	65	M	1	1	2	2	1	178.8	13,350	0.5		c	
94	Sidramappa	19382	72	M	2	2	1	2	1	152.8	11,750	0.6	b		
95	Sahebgouda	19402	67	M	1	2	1	2	2	167	14,020	0.5			a
96	Mdiwalappa`	19001	70	M	2	1	2	1	1	170	15,500	0.4	b		
97	Tarawwa Gireppa	22694	58	F	2	1	2	2	1	161.6	13,000	0.5	b		
98	Prakash	24018	55	M	1	1	2	2	2	176.6	14,140	0.5			a
99	Bhimasingh	24737	63	M	1	2	2	1	1	183.6	14,990	0.5			a
100	Pushpa	24883	60	F	2	1	2	2	1	160.2	13,010	0.5		c	
101	Rajeshwari	24898	55	F	2	2	2	2	1	168	15,590	0.5		c	
102	Shivappa	25171	65	M	1	1	1	2	2	182	17,570	0.4			a
103	Dundawwa Hladgi	25619	60	F	2	1	1	2	2	188	14,500	0.4	b		
104	Sidramappa	25448	65	M	2	1	1	2	2	178.9	13,300	0.5	b		
105	Dathrayya	25388	58	F	2	1	2	1	2	199	15,500	0.5	b		
106	Basvaraj	25396	48	M	1	2	2	2	2	188.6	16,990	0.4			a
107	Gopalchand	25858	65	M	1	1	1	2	2	188.4	15,560	0.4			a
108	Ayub sububalal	25868	48	M	2	2	2	2	1	180	16,190	0.4	b		
109	Bouramma	25906	40	F	2	2	2	2	1	199	15,550	0.4	b		
110	Jnabee	19286	65	F	2	1	2	2	1	221	14,560	0.5		c	
111	Shakuntala	25822	70	F	2	2	1	2	1	186.8	15,300	0.4	b		
112	Shivappa	25171	65	M	1	2	2	1	1	188	14,350	0.5			a
113	Mehaboob saab	25285	70	M	1	1	2	2	2	190	14,560	0.5			a