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Assessment of role of serum bilirubin in the patients diagnosed with coronary artery disease

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Abstract

The development of coronary atherosclerosis is associated with lipid oxidation and generation of free radicals. Several mechanisms have been attributed to anti atherogenic property of bilirubin. The first protective effect of bilirubin relates to the antioxidant property of bilirubin, which prevents lipid oxidation, especially low-density lipoprotein (LDL), and inhibits free radical-induced damages. Bilirubin has proven to be a potent antioxidant under physiological conditions by inhibiting lipid and protein oxidation. Hence based on above data the present study was planned for Assessment of Role of Serum Bilirubin in the Patients Diagnosed with Coronary Artery Disease.

The present study was planned in Department of General Medicine, Indira Gandhi Institute of Medical sciences, Patna, Bihar. In the present study total 50 cases were enrolled. The 25 cases of the Coronary Artery Diseases were evaluated in Group A and remaining 25 control cases were evaluated in Group B. Total bilirubin level in the blood samples of all cases and controls were measured by diazo method (diazotized sulfanilic acid) and with colorimetric technique.

The data generated from the present study concludes that there is an inverse association between serum bilirubin and risk of CAD. Higher serum bilirubin have protective role against CAD, even in the presence of other risk factor. Therefore, Bilirubin level can serve as predictive factor. If confirmed by future retrospective and prospective studies, bilirubin concentrations in conjunction with traditional risk factor could help identify those at high or low risk of CAD.

Keywords: serum bilirubin, coronary artery disease, Bihar, etc

Introduction

Coronary artery disease (CAD), also known as coronary heart disease (CHD) or ischemic heart disease (IHD), involves the reduction of blood flow to the heart muscle due to build-up of plaque in the arteries of the heart. It is the most common of the cardiovascular diseases. Types include stable angina, unstable angina, myocardial infarction, and sudden cardiac death. A common symptom is chest pain or discomfort which may travel into the shoulder, arm, back, neck, or jaw. Occasionally it may feel like heartburn. Usually symptoms occur with exercise or emotional stress, last less than a few minutes, and improve with rest. Shortness of breath may also occur and sometimes no symptoms are present. In many cases, the first sign is a heart attack. Other complications include heart failure or an abnormal heartbeat [1].

Risk factors include high blood pressure, smoking, diabetes, lack of exercise, obesity, high blood cholesterol, poor diet, depression, and excessive alcohol. A number of tests may help with diagnoses including: electrocardiogram, cardiac stress testing, coronary computed tomographic angiography, and coronary angiogram, among others [2].

Ways to reduce CAD risk include eating a healthy diet, regularly exercising, maintaining a healthy weight, and not smoking. Medications for diabetes, high cholesterol, or high blood pressure are sometimes used. There is limited evidence for screening people who are at low risk and do not have symptoms. Treatment involves the same measures as prevention. Additional medications such as antiplatelets (including aspirin), beta blockers, or nitroglycerin may be recommended. Procedures such as percutaneous coronary intervention (PCI) or coronary artery bypass surgery (CABG) may be used in severe disease. In those with stable CAD it is unclear if PCI or CABG in addition to the other treatments improves life expectancy or decreases heart attack risk [3].

In 2015, CAD affected 110 million people and resulted in 8.9 million deaths. It makes up 15.6% of all deaths, making it the most common cause of death globally. The risk of death from CAD for a given age decreased between 1980 and 2010, especially in developed countries. The number of cases of CAD for a given age also decreased between 1990 and 2010. In the United States in 2010, about 20% of those over 65 had CAD, while it was present in 7% of those 45 to 64, and 1.3% of those 18 to 45 [24]; rates were higher among men than women of a given age [4].

Chest pain that occurs regularly with activity, after eating, or at other predictable times is termed stable angina and is associated with narrowings of the arteries of the heart. Angina that changes in intensity, character or frequency is termed unstable. Unstable angina may precede myocardial infarction. In adults who go to the emergency department with an unclear cause of pain, about 30% have pain due to coronary artery disease [5].

Coronary artery disease has a number of well determined risk factors. These include high blood pressure, smoking, diabetes, lack of exercise, obesity, high blood cholesterol, poor diet, depression, family history, and excessive alcohol. About half of cases are linked to genetics. Smoking and obesity are associated with about 36% and 20% of cases, respectively. Smoking just one cigarette per day about doubles the risk of CAD. Lack of exercise has been linked to 7–12% of cases. Exposure to the herbicide Agent Orange may increase risk. Rheumatologic diseases such as

rheumatoid arthritis, systemic lupus erythematosus, psoriasis, and psoriatic arthritis are independent risk factors as well [6].

Job stress appears to play a minor role accounting for about 3% of cases. In one study, women who were free of stress from work life saw an increase in the diameter of their blood vessels, leading to decreased progression of atherosclerosis. In contrast, women who had high levels of work-related stress experienced a decrease in the diameter of their blood vessels and significantly increased disease progression [7]. Having a type A behaviour pattern, a group of personality characteristics including time urgency, competitiveness, hostility, and impatience, is linked to an increased risk of coronary disease.

Limitation of blood flow to the heart causes ischemia (cell starvation secondary to a lack of oxygen) of the heart's muscle cells. The heart's muscle cells may die from lack of oxygen and this is called a myocardial infarction (commonly referred to as a heart attack). It leads to damage, death, and eventual scarring of the heart muscle without regrowth of heart muscle cells. Chronic high-grade narrowing of the coronary arteries can induce transient ischemia which leads to the induction of a ventricular arrhythmia, which may terminate into a dangerous heart rhythm known as ventricular fibrillation, which often leads to death [8].

Typically, coronary artery disease occurs when part of the smooth, elastic lining inside a coronary artery (the arteries that supply blood to the heart muscle) develops atherosclerosis. With atherosclerosis, the artery's lining becomes hardened, stiffened, and accumulates deposits of calcium, fatty lipids, and abnormal inflammatory cells – to form a plaque. Calcium phosphate (hydroxyapatite) deposits in the muscular layer of the blood vessels appear to play a significant role in stiffening the arteries and inducing the early phase of coronary arteriosclerosis. This can be seen in a so-called metastatic mechanism of calciphylaxis as it occurs in chronic kidney disease and hemodialysis (Rainer Liedtke 2008). Although these people suffer from a kidney dysfunction, almost fifty percent of them die due to coronary artery disease. Plaques can be thought of as large "pimples" that protrude into the channel of an artery, causing a partial obstruction to blood flow. People with coronary artery disease might have just one or two plaques, or might have dozens distributed throughout their coronary arteries. A more severe form is chronic total occlusion (CTO) when a coronary artery is completely obstructed for more than 3 months [9].

Cardiac syndrome X is chest pain (angina pectoris) and chest discomfort in people who do not show signs of blockages in the larger coronary arteries of their hearts when an angiogram (coronary angiogram) is being performed. The exact cause of cardiac syndrome X is unknown. Explanations include microvascular dysfunction or epicardial atherosclerosis. For reasons that are not well understood, women are more likely than men to have it; however, hormones and other risk factors unique to women may play a role [10].

Bilirubin is degraded by light. Blood collection tubes containing blood or (especially) serum to be used in bilirubin assays should be protected from illumination. For adults, blood is typically collected by needle from a vein in the arm. In newborns, blood is often collected from a heel stick, a technique that uses a small, sharp blade to cut the skin on the infant's heel and collect a few drops of blood into a small tube. Non-invasive technology is available in some health care facilities that will measure bilirubin by using an instrument placed on the skin (transcutaneous bilirubin meter).

Conjugated bilirubin is often incorrectly called "direct bilirubin" and unconjugated bilirubin is incorrectly called "indirect bilirubin". Direct and indirect refer solely to how compounds are measured or detected in solution. Direct bilirubin is any form of bilirubin which is water-soluble and is available in solution to react with assay reagents; direct bilirubin is often made up largely of conjugated bilirubin, but some unconjugated bilirubin (up to 25%) can still be part of the "direct" bilirubin fraction. Likewise, not all conjugated bilirubin is readily available in solution for reaction or detection (for example, if it is hydrogen bonding with itself) and therefore would not be included in the direct bilirubin fraction.

Total bilirubin (TBIL) measures both BU and BC. Total bilirubin assays work by using surfactants and accelerators (like caffeine) to bring all of the different bilirubin forms into solution where they can react with assay reagents. Total and direct bilirubin levels can be measured from the blood, but indirect bilirubin is calculated from the total and direct bilirubin. Indirect bilirubin is fat-soluble and direct bilirubin is water-soluble [11].

The development of coronary atherosclerosis is associated with lipid oxidation and generation of free radicals. Several mechanisms have been attributed to anti atherogenic property of bilirubin. The first protective effect of bilirubin relates to the antioxidant property of bilirubin, which prevents lipid oxidation, especially low-density lipoprotein (LDL), and inhibits free radical-induced damages. Bilirubin has proven to be a potent antioxidant under physiological conditions by inhibiting lipid and protein oxidation. Hence based on above data the present study was planned for Assessment of Role of Serum Bilirubin in the Patients Diagnosed with Coronary Artery Disease.

Methodology

The present study was planned in Department of General Medicine, Indira Gandhi Institute of Medical sciences, Patna, Bihar. In the present study total 50 cases were enrolled. The 25 cases of the Coronary Artery Diseases were evaluated in Group A and remaining 25 control cases were evaluated in Group B. Total bilirubin level in the blood samples of all cases and controls were measured by diazo method (diazotized sulfanilic acid) and with colorimetric technique.

All the patients were informed consents. The aim and the objective of the present study were conveyed to them. Approval of the institutional ethical committee was taken prior to conduct of this study.

Following was the inclusion and exclusion criteria for the present study.

Inclusion criteria: Cases of Age matched apparently healthy normal subjects free from systemic disease

Exclusion criteria: Cases of haemolytic disease, chronic infection, congenital heart disease, hepatic impairment and antioxidants supplements.

Results & Discussion

Coronary artery disease remains the cause of morbidity and mortality in India; it has already climbed the „charts" from

14th to 4th place only behind tuberculosis communicable disease and malnutrition [12]. In developed countries, coronary heart disease (CHD) is predicted to rise by 30-60% between 1990 and 2020. In developing countries, rates are predicted to increase by 120% in women and by 137% in men from 1920 to 2020 [13]. Bilirubin simply considered as the metabolic end product of heme catabolism that needs to be excreted has emerged as potential endogenous inhibitor of atherosclerosis [14]. It is shown that bilirubin at micromolar concentration in vitro efficiently scavenges peroxyl radicals generated chemically in either homogenous solutions or multilamellar liposomes. Under 2% oxygen, in liposomes bilirubin suppresses oxidation more than α tocopherol, which is regarded as the best antioxidant of lipid peroxidation [15].

Bilirubin has been recognized as a potent antioxidant. Bilirubin suppresses the oxidation of lipid in liposomes more than vitamin E, which is regarded as the best antioxidant of lipid peroxidation. The water-soluble glutathione primarily protects water-soluble proteins, whereas the lipophilic bilirubin protects lipids from oxidation [16]. Serum bilirubin has been demonstrated to be a major contributor to the total antioxidant capacity in blood plasma [17] and proven to have anti-inflammatory properties [18]. Serum bilirubin was shown to be associated with cross-sectional MetS in Chinese children, adolescents, and adults [19, 20] as well as Korean men and women [21, 22]. Patients with Gilbert syndrome whose serum bilirubin levels are high had low levels of oxidative stress associated with enhancement of endotheliumdependent vasodilation [23]. Serum bilirubin has been demonstrated to be negatively associated with cardiovascular disease [24, 26], hemoglobin A1c [27], and albuminuria [28].

Table 1: Basic Details

Parameters	Group A	Group B
Cases of	Coronary Artery Diseases	Control Patients
No. of Cases	25	25
Age in years	48 – 66	51 – 68
Hb (g/dl)	10.8 – 12.7	11.2 – 13.6
Systolic BP (mm of Hg)	101.2 – 137.6	112.6 – 128.3
Diastolic BP (mm of Hg)	65.3 – 81.6	75.9 – 96.7
Body Mass Index (kg/cm2)	21.8 – 27.6	20.1 – 24.3

Table 2: Total Bilirubin

Parameters	Group A	Group B
Cases of	Coronary Artery Diseases	Control Patients
No. of Cases	25	25
Total Bilirubin (mg/dl)	0.42 – 0.63	1.12 – 1.27

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. Bilirubin has proven to be a potent antioxidant under physiological conditions by inhibiting both lipid and protein oxidation. [29] 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 peroxidation19.Additionally, bilirubin exerts anti-inflammatory effects on vasculature and inhibits proliferation of vascular smooth muscle cells [30, 31].

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 [32, 33]. Reduced levels of bilirubin were shown to be associated with higher prevalence of coronary artery disease emerging as new potential risk factor marker.

In India, Veerendra Kumar Arumalla *et al*. [34] 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 [35]. Song YS *et al*. [36] 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 present study verifies an inverse association between the TB concentration and CAD prevalence, in other words bilirubin is diminished in patients with CAD when compared to healthy individuals. Shwertner HA *et al*. [37] were the first to observe an inverse correlation between total circulating bilirubin and occurrence of CAD. They suggested that the relationship of increased CAD risk with low bilirubin was a reflection of its "consumption" of antioxidant activity. Hopkins PN *et al*. [38] also found that the reduction in bilirubin was independent of other known CHD and covariates.

In current scenario bilirubin measurement is only done to assess the effective functioning of liver. The fact that it is anti-oxidative and cytoprotective suggests that it has antiatherogenic properties. As demonstrated, bilirubin has a comparable anti oxidative capacity as vitamin E [39] and alleviates the atherogenic stress of lipid per oxidation by preventing oxidative modification of LDL [40]. It is possible that the protective effects seen with higher bilirubin levels are possibly mediated through hemeoxygenase or by other substrates involved in the pathway of bilirubin production, namely, biliverdin and carbon monoxide [41]. Bilirubin requires vitamin E as the co-oxidant, hence patients with a high bilirubin and deficiency of vitamin E, have less atheroprotective effect that weakens the inverse association between elevated bilirubin levels and the risk of CAD [42].

Bilirubin sub-fractions (Bu and Bc) have demonstrated inhibition of low-density lipoproteins oxidation, which in turn retards the peroxidation of lipids, hence could potentially restrict the progression of atherosclerosis. Increasing pile of evidence suggests that bilirubin is a highly bioactive molecule having deep impact on prognosis of cardiovascular and other diseases. Due to recent discoveries of bilirubin binding to specific nuclear receptors8 (besides PPARα, aryl hydrocarbon receptor (AhR)), and taking into account its production in remote organs, bilirubin behaves in certain circumstances as a hormone.

It is interesting to note that similar endocrine effects have been recently discovered for bile acids, which were also considered for long time as negligible molecules. Pre-

clinical studies have observed this effect to be mediated by preservation of vascular nitric oxide, which mediates endothelial relaxation. Decreased levels of nitric oxide impair the ability of the coronary vessels to dilate during exercise or stress, thus, provoking myocardial ischemia in CAD patients. Besides vaso-relaxation, nitric oxide also inhibits leukocyte adhesion to endothelium, vascular smooth muscle cell migration and proliferation

Conclusion

The data generated from the present study concludes that there is an inverse association between serum bilirubin and risk of CAD. Higher serum bilirubin have protective role against CAD, even in the presence of other risk factor. Therefore, Bilirubin level can serve as predictive factor. If confirmed by future retrospective and prospective studies, bilirubin concentrations in conjunction with traditional risk factor could help identify those at high or low risk of CAD.

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