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ROLE OF SERUM BILIRUBIN IN CLINICALLY ADMITTED PATIENTS TO PREVENT THE RISK OF CARDIAC DISEASES

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Aberrations used:

CVD- Coronary Vascular Disease
CAD- Coronary Artery Disease
ROS- Reactive Oxygen Species,
HbA1C- Glycosylated Hemoglobin
HDL- High Density Lipoprotein
LDL - Low Density Lipoprotein
VLDL- Very Low density lipoprotein
TC- Total Cholesterol
TG- Tri Glyceride

ABSTRACT:

Background: Coronary artery disease /coronary vascular disease (CAD/CVD) remains the first killer and common silent disease in the world. The lipid profile plays the essential role in CAD development with lipid oxidation. Lipid oxidation, which accepted as an important element of arterial plaque formation and atherosclerosis, is involved in the patho physiology of CVD. Bilirubin exerts a strong antioxidant effects at physiological plasma concentrations against lipid oxidation in blood artery.

Methodology: Parameters were measured on a fully automated analyzer using standard reagent kits in a retrospective study involving 100 male Indian subjects between 35 to 55 years of age.

Results: Serum total bilirubin was correlated positively with serum high density lipoprotein (HDL), while it was negatively correlated with glycosylated hemoglobin (HbA_1c), there were no statically significance present between serum total cholesterol and serum triglyceride in hospitalized patients, not in healthy subjects.

Conclusions: we were concluded that, the serum total bilirubin concentration will be an independent cardiovascular risk factor such as lipid profile.

INTRODUCTION: Bilirubin is considered a strong reducing agent and a potential physiological antioxidant [1]. The main role of bilirubin antioxidation action is via ROS process, the ROS of biliverdin and bilirubin generation are both potent scavengers of peroxyl radicals [2].

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The free and albumin-bound bilirubin is able to reduce O^- and inhibit plasma LDL lipid peroxidation [3]. Different circulation forms of bilirubin, which are acting powerful antioxidants: free bilirubin, albumin-bound bilirubin, conjugated bilirubin (free bilirubin is conjugated to either glucuronic acid or sulfate), and unconjugated bilirubin. All are effective as scavengers to peroxyl radicals and have the ability to protect human LDL against peroxidation [4]. Bilirubin and more especially albumin-bound bilirubin are found to be cytoprotective to human erythrocytes and human myocytes during cells exposed to oxyradicals [5].

Antioxidant activity and cardioprotective potential may be attributable to any of the bilirubin forms, including free unconjugated bilirubin, protein-bound unconjugated bilirubin, delta bilirubin, or mono-/diconjugated bilirubin [6]. Hence, bilirubin production is involved in antioxidant defense mechanisms and that higher bilirubin concentrations are associated with a lower incidence of oxygen radical-mediated injury [7, 8, 9]. Low concentrations of serum bilirubin are associated with increased risk of IHD [9].

Lipid profile (Cholesterol, Triglyceride, and LDL) are the essential players compounds in CAD developments steps, except HDL which retards CAD development. According to other studies of lipid profile with CAD relationship, multiple epidemiologic studies have established a low level of HDL as an independent risk factor for CVD [10].

Abnormalities of the oxidation-reduction state of LDL in the vessel wall may be an important pathogenic mechanism in atherosclerosis [11], which lead to start the atherogenesis process in layers of vessel wall.

The Framingham Heart Study observed that the individuals with HDL concentrations of ≥ 60 mg/dL are protected against the development of CAD even in the presence of elevated serum LDL levels [12]. In general, the HDL and its components associated (including apo A-I, paraoxonase, platelet activating factor acetylhydrolase, and other antioxidant enzymes) exert an array of effects that may help prevent atherosclerosis and acute coronary syndromes [13].

The oxidative reactions are involved in the pathophysiology of disease processes[14.] According to the literatures, serum Bilirubin had an inverse relationship with CAD risk factors as serum cholesterol, cigarettes smoked/day, high systolic blood pressure, serum TG and fasting glucose [15]. But little literature had not observed any relationship between bilirubin and lipid profile [16].

In view of aforementioned controversial literature, we were aimed to evaluate the different biochemical lipid parameters with HbA_1C, related to risk of CVD/ CAD and serum Bilirubin, in the healthy and admitted subjects.

MATERIALS AND METHODS: This retrospective study was conducted at the Shri Ram Murti Smarak Institute of Medical Sciences, Bareilly (India) on 100 male [50 healthy OPD and 50 IPD admitted in the hospital for clinically different complaints instead of CVD/CHD] subjects. The clinical biochemistry laboratory of the hospital of Shri Ram Murti Smarak Institute of Medical Sciences, Bareilly (north Indian city) regularly analyses samples of company employees who attend the medicine outpatient department (OPD) for "health checkups" under which they are tested for various biochemical parameters including HbA_1C , serum bilirubin and the standard lipid profile. Such OPD and IPD attendees from March 2012 to October 2012, which were tested for HbA_1C, lipid profile, and serum bilirubin, were considered for our study. The exclusion criteria were (a) females, (b) men below 35 or above 55years and (c) subjects who had TG > 400 mg/dl.(d) patients on known lipid altering medications, (e) diabetics, patients with chronic kidney disease, liver disease and CVD. As per hospital records, all the selected "health check-up patients" were healthy by normal standards and had no associated cardiovascular complications. The lipid profile, HbA_1c and serum Bilirubin levels of these subjects were used for our study.

Blood Sampling and Routine Biochemical Analysis: Serum was separated from venous blood of fasting Subjects and analyzed within two hours of collection.Serum Bilirubin, HbA_1C, serum TG and serum TC were analyzed By DCA, Enzymatic , GPO-PAP and CHOD-PAP methods respectively on fully automated analyzer of mind ray series . Serum HDL-C was measured using reagent kit (Accurex, Mumbai) on semi-autoanalyzer- BTR-830 (Biosystems, SA, Spain). This uses the supernatant for HDL-C assay by the same enzymatic method used for TC analysis, after the other lipoproteins are precipitated by phosphotungstate and Mg^{2+}. VLDL-C and LDL-C were calculated by the standard formula (Friedewald *et al.*, 1972) as TG was < 400 mg/dl in the selected subjects. Results are presented as mean ± SD. A p value < 0.05 was considered significant. Statistical analysis was performed using GraphPad Prism version 5.00 for Windows (GraphPad software, San Diego California USA, www.graphpad.com).

RESULTS: Several biochemical processes participating in CAD development, include lipid and apo lipoprotein metabolism, inflammatory response, endothelial function, platelets function, thrombosis, fibrinolysis, and blood pressure regulation[17]. In our study Table represented the biochemical lipid parameters, with serum total bilirubin.

Table 1 showed a great difference between OPD and IPD (32.78 ± 4.44, 50.34 ± 6.57) subjects in serum HDL level, respectively. In the same manner this table also showed a higher level of HbA_1C (5.38 ± 0.92) in OPD subjects. It was represented that the OPD subjects were more prone to risk of CVD. While in IPD subjects it was represented a lower level of (4.54 ± 0.40) HbA_1C.

TABLE 1: DIFFERENT LIPID AND BIOCHEMICAL PARAMETERS IN HEALTH CHECK (OPD) AND ADMITTED (IPD) SUBJECTS

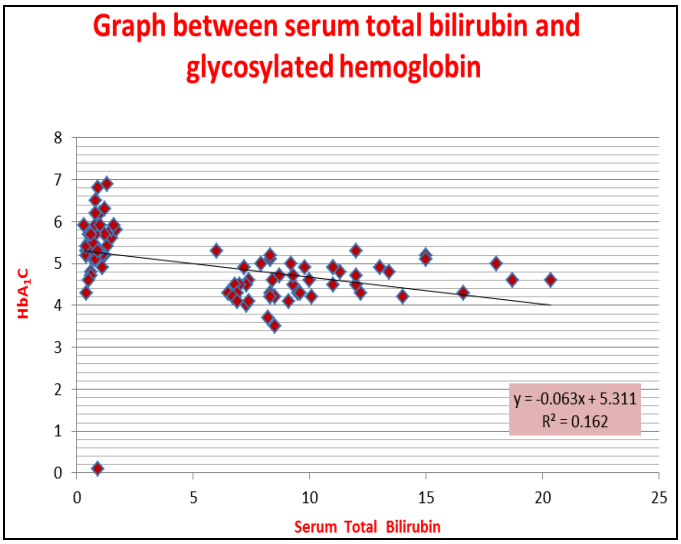
Subjects Group	Serum Total cholesterol (TC) Mean±SD	Serum Total Triglyceride (TG) Mean±SD	Serum High Density Lipoprotein (HDL) Mean±SD	Serum Low Density Lipoprotein (LDL) Mean±SD	Serum Total Bilirubin (TB) Mean±SD	Serum Direct Bilirubin (DB) Mean±SD	Serum Indirect Bilirubin (IDB) Mean±SD	Glycosylated hemoglobin (HbA_1C) Mean±SD
OPD Subjects	217.80±44.26	113.30±24.77	32.78±4.44	162.36±46.69	0.812±0.34	0.30±0.20	0.50±0.30	5.38± 0.92
IPD Subjects	217.94±41.76	114.54±26.92	50.34±6.57	144.69±43.47	10.08±3.3	6.37±2.56	3.71± 1.88	4.54 ±0.40
SEM OPD	6.26	3.81	0.63	6.60	0.049	0.02	0.04	0.13
SEM IPD	5.91	3.81	0.93	6.14	0.47	0.36	0.26	0.05
p-value	0.9871	0.8111	0.0001*	0.0531	0.0001*	0.0001*	0.0001*	0.0001*
	Non-significant	**Non-significant**	**Significant**	**Non-significant**	**Significant**	**Significant**	**Significant**	**Significant**

*Significant p- value; SEM – standard error of mean, OPD – outdoor patients department, IPD- indoor patients department

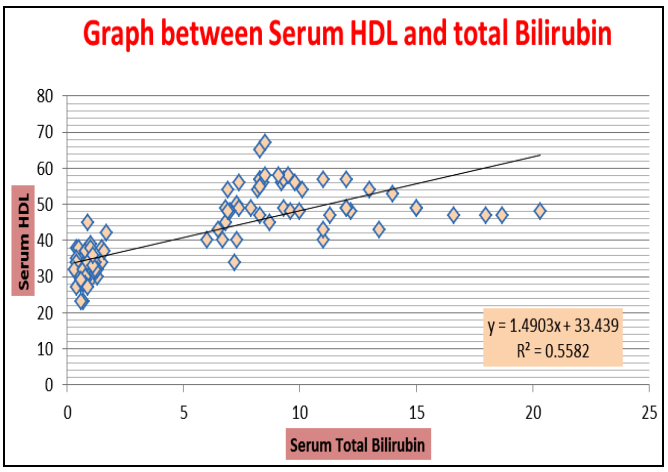
Table showed there were no statically significant differences between serum TC and serum TG (217.80 ± 44.26; 217.94 ± 41.76 and 113.30 ± 24.77; 114.54 ± 26.92) in both groups, respectively. Along with this there were found no statically significant differences in serum LDL in both groups.

Graph A represented the relation between HbA_1C and serum total bilirubin. According to this graph in the OPD subjects increased the level of HbA_1C when decreased the level of serum total bilirubin, while in the IPD patients decreased the level of HbA_1C when increased the level of serum total bilirubin.

Graph B showed a relation between serum HDL and total bilirubin in OPD and IPD subjects. According to this graph when increased the level of serum total bilirubin then increased serum HDL level in IPD subjects, while in OPD subjects when bilirubin level remain normal there were no change in serum HDL level, it represented that higher serum bilirubin was directly correlated with higher HDL level or it was inversely correlated with risk of CVD/ CHD in admitted patients.



GRAPH A: BETWEEN SERUM TOTAL BILIRUBIN AND GLYCOSYLATED HEMOGLOBIN IN OPD AND IPD SUBJECTS



GRAPH B: BETWEEN SERUM TOTAL BILIRUBIN AND SERUM HDL LEVEL IN OPD AND IPD SUBJECTS

DISCUSSION: In our study, we were found a positive relation in admitted patients, between serum total Bilirubin concentration and serum HDL, while there were no relation between serum HDL concentration and serum Bilirubin level in OPD patients, who had normal Bilirubin concentration. It means when increased the level of serum total Bilirubin in admitted patients at the same decreased the risk of CVD and CAD in these patients, while OPD patients with normal Bilirubin concentration had higher risk of CVD/CAD.

Coronary artery diseases develops through narrowing of the coronary arteries which leads to death of portion of the heart muscle because of lacking of blood flow that supply oxygen and nutrition, and leads to heart attack.[18]. Lipid profile plays the essential role of lipid deposition in artery wall and CAD development, by accumulating the LDL inside layers of artery wall, except HDL which has beneficial effects for a number of reasons by decreasing lipid oxidation after depositing in blood vessels, leading to retarding CAD development [19, 20].

Serum Bilirubin is derived primarily from the degradation of hemoglobin. Bilirubin exerts a strong antioxidant effects at physiological plasma concentrations against lipid oxidation in blood artery (1). While the heme oxygenase (HO^{-1}) is a key enzyme of heme catabolism from red blood cells breakdown, which catalyzes the oxidative cleavage of heme which results in releasing CO, iron (Fe^{2+}), and Bilirubin.

An inducible form of HO (HO^{-1}) is expressed at a low concentration in vascular endothelial and smooth muscle cells. It is markedly induced by heme, metals oxidative stress, inflammatory mediators oxidized LDL and hypoxia. Several experiments have suggested that HO-1 is a stress response protein that plays an important function in cell defense mechanisms against oxidative injury. HO-1 activity is responsible for increased CO and Bilirubin formation as well as iron release in pathological conditions such as CVD, hypoxia, ischemic-reperfusion and hypertension[21].

According to literatures each 1-mg/dL decrease in plasma HDL concentration is associated with a 2% to 3% increased risk of CVD[18, 19]. It means when increased the level of serum HDL then decreased the risk of CVD/ CAD. A low level of HDL as an independent risk factor for CVD [10]. Our study was also correlated with Framingham Heart Study which was reported 43% to 44% of coronary events occurred in persons with HDL levels less than 40 mg/dL[22]. The beneficial effects of HDL are via protection through multiple pathways, which is including both reverse cholesterol transport and non-cholesterol dependent mechanisms [23].

High-normal plasma level of Bilirubin was reported to be inversely related to atherogenic risk and to provide protection against endothelial damage. Risk reduction by bilirubin was comparable to that of HDL by CAD development retarding mechanism[14, 24]. The oxidative stress was found to cause depletion of endogenous antioxidants, including bilirubin, in human plasma and to increase production of lipid hydro peroxides [25]. In addition, other study has shown that the increases in serum bilirubin concentration (but still within the normal range) are associated with a significant and marked reduction in CAD risks [8]. Few studies concluded that low concentrations of serum bilirubin are associated with increased risk of IHD [9].

The relation of bilirubin with CAD risk factors was described by other investigators who found that plasma bilirubin correlated inversely with several known risk factors for CAD, such as smoking, LDL-cholesterol, DM, and obesity, and correlated directly with the protective factor HDL-cholesterol [26].

In our study we were not found any statically significant differences between serum TC, TG and LDL in both groups. But we were found a negative relation between serum bilirubin and glycosylated hemoglobin in admitted patients, while there were a positive relation in OPD patients who had normal or lower Bilirubin concentration. In middle-aged adults, higher "normal" levels of glycated hemoglobin were associated with increased risk for diabetes, CVD, ischemic stroke, and total mortality in adults [27]. This fact was also correlated to our study with the fact of inverse association between serum bilirubin and CAD development, although it involves endogenous anti-oxidant byproduct as HDL role.

CONCLUSIONS: According to this retrospective study we were found that Most of admitted subjects had higher serum HDL level with higher serum total Bilirubin, while there were no significant changes in serum TC, TG and LDL level in comparisons to healthy subjects. According to this we were concluded that admitted patients with higher serum total Bilirubin concentration had a lower risk of CVD/CAD in comparison to OPD subjects.

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