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Serum bilirubin levels are negatively associated with atherogenic lipids in Saudi subjects with type 2 diabetes: A pilot study

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ABSTRACT

Background: Recent research has demonstrated the possible relevance of bilirubin in metabolic and cardiovascular disorders. Lipid abnormalities are a major problem that is related with an increased risk of cardiovascular disease in diabetics. This study examined the relationship between serum bilirubin and direct bilirubin concentrations and atherogenic lipids in patients with type 2 diabetes (T2DM).

Methods: This cross-sectional included 67 patients with type 2 diabetes and 39 matched healthy control. The lipid profile, including total cholesterol, HDL-C, and TG levels, fasting blood glucose, total bilirubin, direct bilirubin, ALT, AST, and ALP were measured using a dimension EXL clinical chemistry analyzer (Siemens Healthcare Diagnostics). Cholesterol in VLDL, LDL, and sdLDL were calculated from standard lipid assay results by the equations of Sampson et al.

Results: Serum bilirubin was lower in non T2DM subjects nearly significant (p=0.0.51) whereas direct bilirubin concentrations were lower in T2DM (p=0.008). ALT, AST, and ALP levels were higher in T2DM groups. The mean values of LDL-C, sdLDL-C, non HDL-C and VLDL-C were significantly increased in T2DM group and lower HDL-C. An inverse relationship could be observed with increase in serum total bilirubin and serum levels of LDL-C (r^2=0.139, p<0.005), sdLDL-C (r^2=0.137, p<0.005), VLDL-C (r^2=0.074, p<0.044), and non HDL-C (r^2=0.166, p<0.002) in T2DM group. The same inverse relationship was observed with serum direct bilirubin and serum levels of LDL-C (r^2=0.133, p<0.006), sdLDL-C (r^2=0.172, p<0.001), VLDL-C (r^2=0.118, p<0.01), and non HDL-C (r^2=0.182, p<0.001) in T2DM group.

Conclusions: A significant negative association was found between serum bilirubin levels and direct serum bilirubin with atherogenic lipids, suggesting that serum bilirubin may protect T2DM patients from development of cardiovascular disease. These findings indicate the need for additional research in a large cohort.

Keywords: atherogenic, non-HDL cholesterol, small dense LDL, bilirubin, type 2 diabetes, direct bilirubin

INTRODUCTION

Bilirubin is heme's end product breakdown resulted from sequential enzymatic activity of heme oxygenase and biliverdin reductase [1]. Apart from its role as a serum marker of hepatic disorders, bilirubin has powerful antioxidant characteristics, as indicated by its capacity to scavenge peroxyl radicals and limit the oxidation of low-density lipoprotein (LDL) [2]. Bilirubin at an adequate increased level has been shown to be advantageous in a number of investigations. Bilirubin is adversely linked with C-reactive protein sensitivity, glycated hemoglobin, and type 2 diabetes mellitus (T2DM) [3, 4].

T2DM is a chronic metabolic condition that has become a major source of morbidity and medical costs [5]. Globally, the number of individuals living with diabetes has risen substantially. In 2014, it was projected that 536.6 million people worldwide had diabetes; by 2045, that figure is expected to rise to 700 million, with 80 percent of them living in low- and middle-income nations [5, 6]. Patients with T2DM are at higher risk for number of life-threatening health problems. Lipid abnormalities are a serious issue occurs more commonly in diabetics, associated with the increased risk of cardiovascular disease (CVD) [7].

In these individuals, the most common pattern of dyslipidemia consists of high triglyceride levels and low high-density lipoprotein cholesterol levels [8]. Low density lipoprotein (LDL) is divided into several subclasses, each with its own size, density, physicochemical makeup, metabolic behavior, and atherogenicity [9]. Changes in LDL structure caused by oxidation, enzymatic degradation, or lipolysis may hasten the progression of atherosclerosis [10]. Atherogenic lipids include hypertriglyceridemia, elevated sd LDL particles, reduced HDL cholesterol and elevated HDL particle numbers, elevated remnant lipoproteins, and postprandial hyperlipidemia [11]. In compared to big particles, Atherogenicity is expected to be higher in sdLDL particles [12, 13]. The presence of high quantities of sdLDL particles has been linked to coronary artery disease. Elevated small dense LDL (sdLDL) levels have been linked to an increased risk of ischemic heart disease (IHD) in numerous investigations [14]. Several studies have linked sdLDL to coronary artery disease, and it is now thought that sdLDL particles are a powerful predictor of cardiovascular events and coronary artery disease

development [13]. Moreover, sdLDL-cholesterol has recently been acknowledged as a promising diagnostic test for assessing heart disease risk [15]. The purpose of this research was to examine the relationship between serum bilirubin and direct bilirubin concentrations and atherogenic lipids in type 2 diabetes mellitus.

MATERIALS AND METHODS

This cross-sectional study comprised 67 individuals with type 2 diabetes, ranging in age from 14 to 77 years (35 men and 32 women). Seven ml of fasting blood samples were taken, centrifuged at 3,000 g for 15 minutes, and then serum or plasma was separated. After 10 to 12 hours of fasting, blood samples were drawn into serum separator tubes for analysis of fasting blood glucose and lipid profiles. The samples were transferred under controlled circumstances to the main laboratory, where they were promptly centrifuged and evaluated. Using a dimension EXL clinical chemistry analyzer, the lipid profile, including total cholesterol, HDL-C, and TG levels, fasting blood glucose, total bilirubin, direct bilirubin, ALT, AST, and ALP were analyzed (Siemens Healthcare Diagnostics). Cholesterol in VLDL, LDL, and sdLDL was estimated using the equations based on the findings of a routine lipid test [16].

Preventive maintenance, function checks, calibration, and quality control were used in accordance with the manufacturer's instructions to regulate the analytical procedures. All samples were subjected to automated interference analysis for the detection of hemolysis, icterus, and turbidity.

Statistics

The statistical significance of the differences between means as determined by data is given as mean ± standard deviation. For group comparisons, the student unpaired t-test was performed for data with a normal distribution, whereas the Mann-Whitney test was performed for data with an abnormal distribution. To explore various associations, Pearson's correlation test was employed. Significant differences were determined to exist when p-value was less than 0.05 ($p<0.05$).

RESULTS

67 T2DM patients and 53 non-diabetic participants were selected for the study. **Table 1** displays their clinical and laboratory features. Age and gender distributions did not differ substantially (p=0.342) between individuals with and without T2DM (**Table 1**). 49% of the 106 participants were female. In the T2DM group, the mean fasting blood glucose concentration was 9.93 (±3.94) mmol/L, while in the control group it was 4.82 (±0.44). Mean HbA1c levels were 8.37% (±1.15%) in those with T2DM and 5.19% (±0.85%) in the control group. T2DM was diagnosed due to higher levels of fasting glucose and HbA1c (**Table 1**). Serum bilirubin was lower in non T2DM subjects nearly significant (p=0.0.51) whereas D-bil whereas D-bil concentrations were lower in T2DM (p=0.008). ALT, AST, and ALP levels were higher in T2DM groups (**Table 1**). The mean values of LDL-C, sd LDL-C, non HDL-C and VLDL-C were significantly increased in T2DM group and lower HDL-C as shown in **Table 1**.

Table 1. Clinical and laboratory characteristics of patients

	Control (n:39)	T2DM (n:67)	p-value
Age (years)	39.10±17.00	53.60±12.10	0.3420
Gender	20 F/19 M	32 F/35 M	
FBG (mmol/L)	4.82±0.44	9.93±3.94	<0.0001
HbA1c (%)	5.19±0.85	8.37±1.15	<0.0001
TC, mg/dL	171.04±32.28	173.47±42.20	0.6810
HDL-C, mg/dL	53.61±14.41	41.16±9.81	<0.0001
LDL-C, mg/dL	101.42±16.38	105.71±37.10	0.4150
sdLDL-C, mg/dL	27.63±6.07	35.85±12.37	<0.0001
VLDL-C, mg/dL	14.18±5.15	25.02±13.93	<0.0001
Non HDL-C, mg/dL	117.43±25.30	132.31±41.07	0.0110
T bil, µmol/L	6.79±3.40	8.45±4.70	0.0510
D bil, µmol/L	2.58±1.04	2.00±0.98	0.0080
ALT, IU/L	19.33±10.09	34.82±17.55	<0.0001
AST, IU/L	17.60±4.78	19.72±13.60	0.2670
ALP, IU/L	72.48±16.91	78.75±31.32	0.1940

Note. ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; FBG: Fasting blood glucose; D bil: Direct bilirubin; T bil: Total bilirubin; TC: Total cholesterol; TGs: Triglyceride; HDL-C: High density lipoprotein cholesterol; LDL-C: Low density lipoprotein cholesterol; sd LDL-C: Small dense low density lipoprotein cholesterol; non-HDL-C: Non-high density lipoprotein cholesterol; & VLDL-C: Very low-density lipoprotein cholesterol

An inverse relationship could be observed with increase in serum total bilirubin and serum levels of LDL-C (r^2=0.139, p<0.005), sd LDL-C (r^2=0.137, p<0.005), VLDL-C (r^2=0.074, p<0.044), and non HDL-C (r^2=0.166, p<0.002) in T2DM group, as shown in **Figure 1**, A, B, and D, respectively. The same inverse relationship was observed with serum direct bilirubin and serum levels of LDL-C (r^2=0.133, p<0.006), sd LDL-C (r^2=0.172, p<0.001), VLDL-C (r^2=0.118, p<0.01), and non HDL-C (r^2=0.182, p<0.001) in T2DM group, as shown in **Figure 2**, A, B, and D respectively. Whereas in healthy control there is no significant relationship was observed with both serum total bilirubin or serum direct bilirubin and serum levels of LDL-C, sd LDL-C, VLDL-C, and non HDL-C as shown in **Figure 1** and **Figure 2**, respectively.

DISCUSSION

The present cross-sectional study clearly established a significant inverse association between total serum bilirubin and direct serum bilirubin and atherogenic lipids, suggesting that the highest serum bilirubin levels may play a protective role against incident CHD and CVD.

The findings in this study are in line with previous studies that have reported negative relationship between serum bilirubin and the risk of CVD. Schwertner et al was the first study to report such relationship [17]. In a cross-sectional assessment of a non-diabetic population, the researchers discovered a significant inverse relationship between bilirubin levels and the occurrence of coronary artery disease [17]. Lipids and lipoprotein particles play a critical role in atherosclerosis, the underlying pathophysiology of cardiovascular disease, by influencing inflammatory processes as well as the activity of leukocytes, vascular, and cardiac cells, all of which have an effect on the arteries and heart [18, 19]. Atherogenic lipid profile seems to be common in T2DM patents. Atherogenic dyslipidaemia includes both quantitative and qualitative lipoproteins abnormalities.

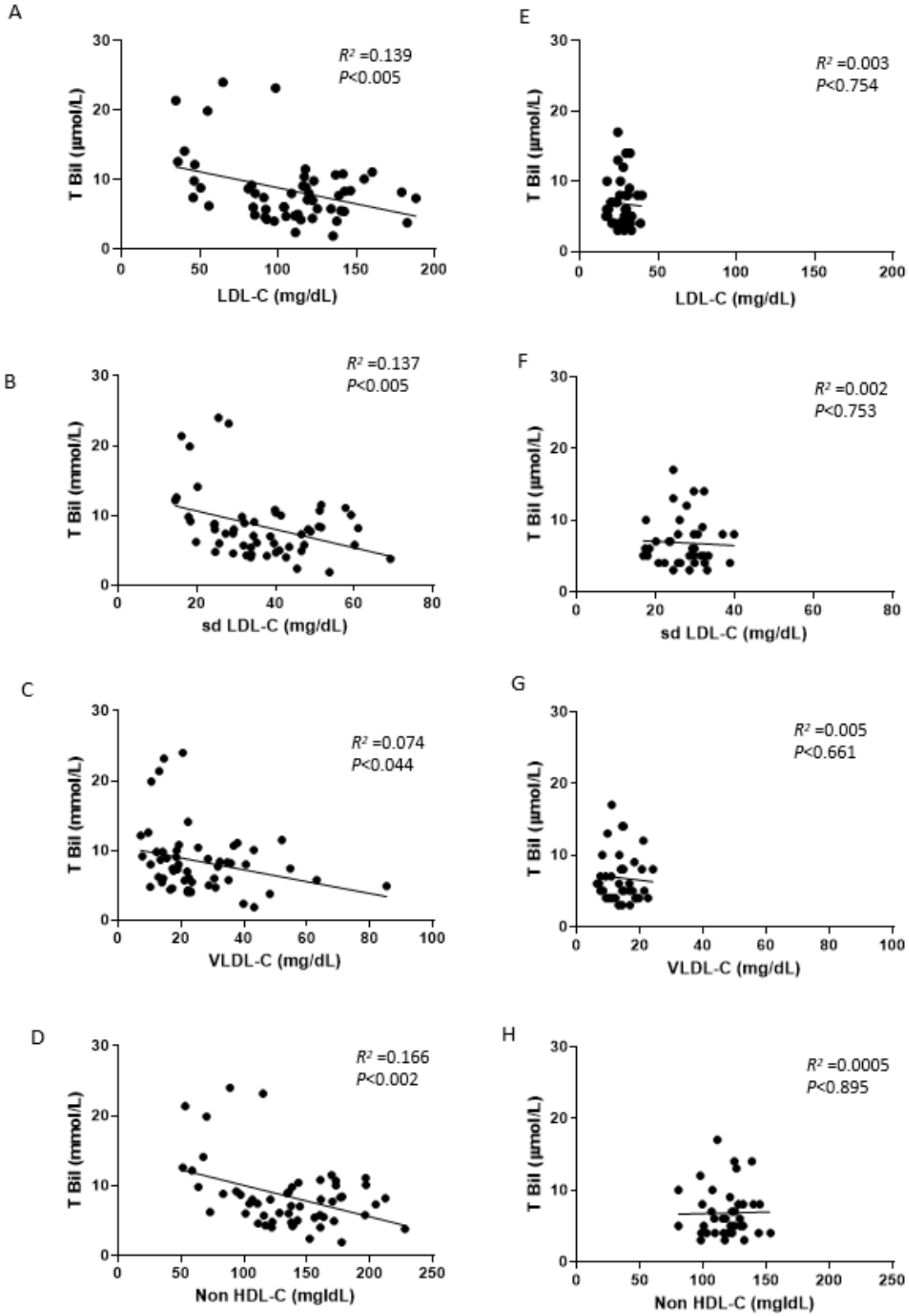

Figure 1. Relationships of plasma LDL-c, sd LDL-C, VLDL-C, and non HDL-C with serum total bilirubin in 67 subjects with type 2 diabetes mellitus (T2DM) (A, B, C, and D, respectively) and in 39 non-diabetic subjects (E, F, G, and H, respectively) (Source: Author's own elaboration)

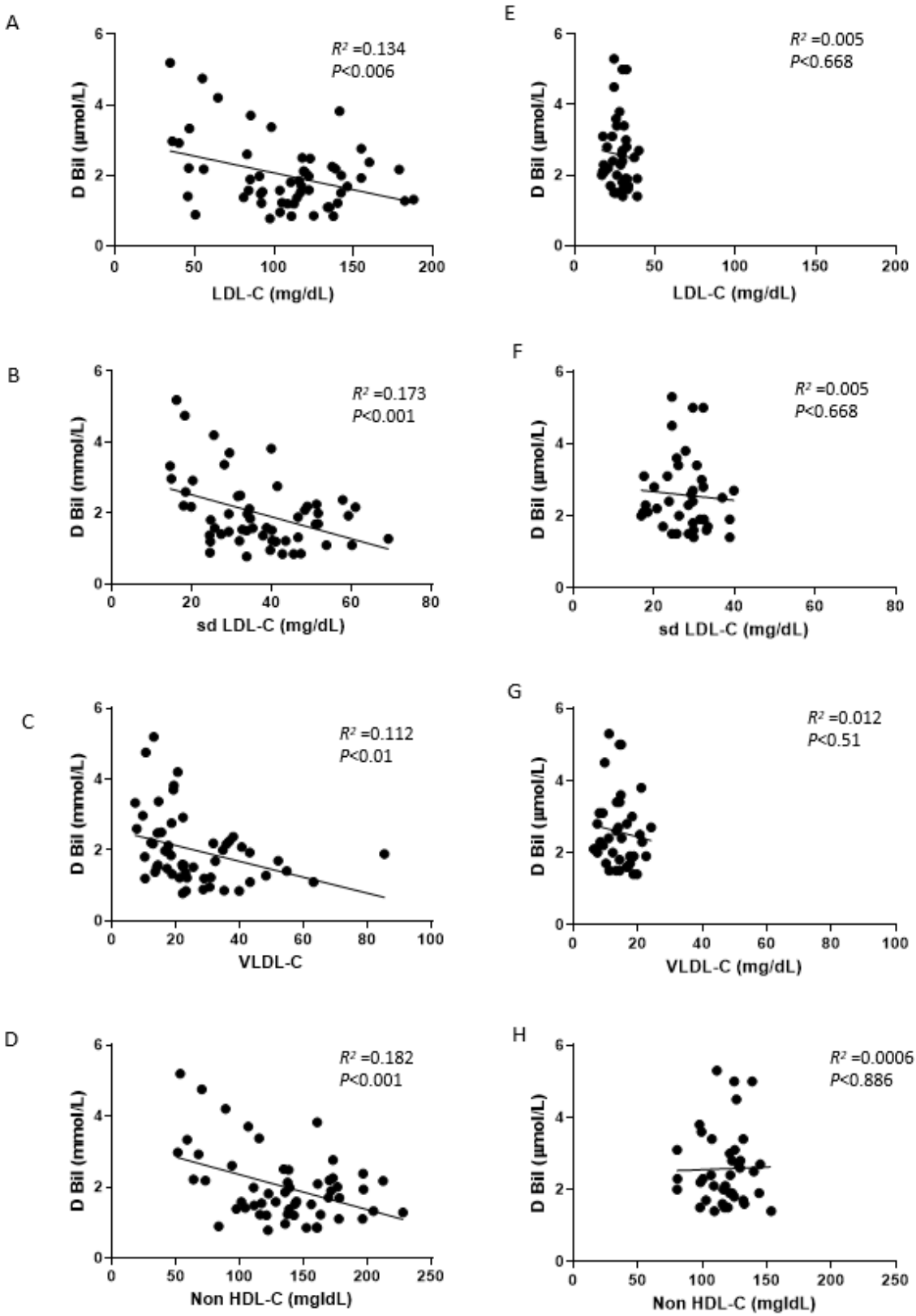

Figure 2. Relationships of plasma LDL-c, sd LDL-C, VLDL-C, and non HDL-C with serum direct bilirubin in 67 subjects with type 2 diabetes mellitus (A, B, C, and D, respectively) and in 39 non-diabetic subjects (E, F, G, and H, respectively) (Source: Author's own elaboration)

Quantitative lipoproteins abnormalities characterized by hypertriglyceridemia, increased residual particle levels (due to an increase in the synthesis of triglyceride-rich lipoproteins and a decrease in the rate of catabolism of triglyceride -rich lipoproteins), and lower HDL-cholesterol levels due to an increase in HDL catabolism. While qualitative lipoprotein abnormalities that are more atherogenic are defined by an increase in big VLDL particle size (VLDL1), an increase in the fraction of small dense LDL particles, an increase in the triglyceride content of both LDL and HDL, and apolipoprotein glycation [7, 20].

It has been showed that high levels of circulating LDL-C concentrations are considered a major risk factor for coronary heart disease, with each 1.0 mmol/l drop reducing the incidence by 10%-20% [21]. Despite a considerable reduction in LDL-C by medication, a large reservoir of cardiovascular disease risk persists. Several metabolic diseases have been considered as potential contributors to the residual risk. One such condition is an excess of small, dense LDL particles, which has been studied since the 1950s and whose therapeutic significance as a nontraditional marker of CHD risk has been supported by recent clinical trial findings [22]. T2DM group in this study show no significant increase circulating LDL-C concentrations in comparison with control group and this can be attributed to statin use [21]. However, serum sdLDL level was found to be significantly increased in the T2DM group as compared to the healthy control. It was observed increased sdLDL in patients who were treated with statins [23].

The data of this study showed a strong inverse relationship between both serum bilirubin and direct bilirubin with sdLDL. Patents with Gilbert's syndrome, which is characterized by a benign, mildly elevated serum bilirubin have reduced serum levels of atherogenic lipids [24]. The protective role of bilirubin in T2DM is primarily attributed to the antioxidant activity of bilirubin [25]. Many studies showed that, within physiological ranges, total bilirubin can inhibit hyperglycemia-induced free radical damage to cells [13, 26].

It was found that level of total bilirubin in the serum was shown to be inversely related to the presence of stroke, and people with a history of stroke and greater bilirubin levels were less likely to have had a worse stroke outcome [27]. However, It has been noted that abnormal high levels of serum total bilirubin caused by abnormal liver function resulted in unprotected for CVD [28].

It has been suggested that the mechanism by which higher serum total bilirubin may contributes to reduced CVD risk include through antioxidant activities, anti-inflammatory effects, antiatherogenic properties, or through pathways that involved in vascular structure and reactivity [29]. One study showed that unconjugated bilirubin, at normal blood concentrations, was acted as an excellent scavenger of singlet oxygen molecules, disrupting free radical chain reactions and functioning as an antioxidant. This activity spikes as the experimental settings shift from ambient oxygen concentrations to very low tissue oxygen concentrations [1].

In addition, bilirubin can protect vascular endothelial cells from oxidative stress [30]. Animal studies also have shown that biliverdin, a precursor of bilirubin, prevents impaired glucose tolerance [31]. A recently publishes study showed that Bilirubin contributed to total antioxidant capacity and is negatively associated with serum markers of oxidative stress [32]. These results explain the biological basis for the inverse association between serum bilirubin and type 2 diabetes. Therefore, the decreased prevalence of diabetes may be attributable to bilirubin's antioxidant function.

This study also demonstrates that T2DM groups had elevated levels of non-HDL cholesterol. Non-HDL cholesterol associated strongly with Hb A1c. Non-HDL cholesterol levels were regarded as an additional technique for assessing cardiovascular risk in individuals whose cardiovascular risk is not adequately diagnosed by LDL cholesterol alone. Non-HDL cholesterol evaluates apo B-containing lipoproteins that indicate atherogenic lipid levels [33]. Non-HDL cholesterol measurement is advantageous and cost-effective since it does not need a 12-hour fast, which poses a risk for hypoglycemia in T2DM patients [34]. NCEP's adult treatment panel III acknowledged the importance of non-HDL cholesterol in diabetes and saw it as a secondary therapeutic target [35]. Even if LDL cholesterol levels are at or below NCEP objective or seem normal in T2DM, elevated non-HDL cholesterol levels have been linked to an increased risk of cardiovascular disease [36].

There are a number of limitations that must be highlighted in this study. To begin with, only a small number of patients were investigated because the primary aim was detailed metabolic investigation. In addition, a lack of information on the drug types and patients' adherence to these medications for diabetes, hypertension, and dyslipidemia may make it difficult to interpret the results. Finally, because the data in this study is cross-sectional, it is unable to find a clear cause of the association between dyslipidemia and serum bilirubin in diabetic patients.

In conclusion, analyzed data from this pilot study has provided evidence that increased serum levels of bilirubin and direct bilirubin are negatively associated with atherogenic lipids in type 2 diabetic patients, which suggests that the serum bilirubin levels may be a biomarker of CVD progression.

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Ethical statement: Research Ethics Committee, College of Medicine, University of Hail authorized the research protocol (HREC 00084/CM-UOH.12/19). Before enrollment, written informed permission was collected from all individuals.

Declaration of interest: No conflict of interest is declared by the author.

Data sharing statement: Data supporting the findings and conclusions are available upon request from the author.

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