GLYCATED LOW-DENSITY LIPOPROTEIN IN DIABETIC AND NON-DIABETIC PATIENTS

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ABSTRACT

Objective: The importance of measuring the blood level of modified low-density lipoprotein (LDL) molecules is an effective method of identifying people at risk of coronary atherosclerosis; this is because, in the early stages of atherosclerosis, lipolysis and oxidative modification have a role in promoting the uptake of these lipids through macrophages; therefore, this research aims to measure the level of glycated LDL (Gly-LDL) in the blood and its association with metabolic parameters of diabetic patients (diabetes mellitus) and non-diabetic (hyperlipidemia).

Methods: At a University Diabetes Center in Riyadh, we used routine automatic analysis methods, fasting serum samples were analyzed for 31 patients with Type-2 diabetes and 31 non-diabetic patients for LDL, high-density lipoprotein (HDL), total cholesterol, glycated hemoglobin, glucose, and triglycerides (TG) and using enzyme-linked immunosorbent assay to analyze Gly-LDL for the same sample.

Results: The serum level of Gly-LDL in non-diabetic was higher than in diabetic patients (p=0.037), Gly-LDL level correlated significantly with LDL in the diabetic group (p=0.035) and was insignificant with other parameters; moreover, it is significantly correlated with HDL (p=0.048), TG (p=0.035), and very LDL (p=0.03) in the non-diabetic group and insignificant with other parameters.

Conclusion: Measuring rates of Gly-LDL can be used in the early detection of cardiovascular disease, especially in people with diabetes, as they are more susceptible to modified and oxidized LDL.

Keywords: Hyperlipidemia, Low-density lipoprotein, Glycated low-density lipoprotein, Diabetes mellitus, Atherosclerosis.

INTRODUCTION

Type 1 and Type 2 diabetes are among the most severe cases of coronary artery disease, peripheral arterial disease, and additional stroke. One of the most important causes of diabetes complications is prolonged hyperglycemia [1]. The risk of hyperglycemia is that it induces a large number of vascular tissues that can promote atherosclerosis [2], although diabetes mellitus (DM) accelerated mechanisms of atherosclerosis are considered vague [3]. The presence of high levels of glucose in DM patients leads to the formation of lipids and conditions favorable to non-enzymatic change of proteins and forming advanced glycation end products [4]. The results of the study showed that non-diabetic patients taking statins had lower serum levels of glycated low-density lipoprotein (Gly-LDL); this confirms that Gly-LDL occurs in all non-diabetic patients regardless of their status of glycemic [5].

Gly-LDL produced when glucose forms a covalent bond with the lysine residues of the apolipoprotein B (Apo B100), the main apolipoprotein of LDL, which is present in both diabetic and non-diabetic subjects, although it is higher in diabetic subjects more than non-diabetics ones [6].

The glycation of lipoproteins was initially reported by Schleicher et al. in 1981 [7]. Concerning normolipemic diabetic patients confirmed with some of the presence of LDL glycation with glycemic control. In the view of Singh et al., they emphasized that glycated lipoproteins (LDL, high-density lipoprotein [HDL]), and very LDL (VLDL) contribute to atherosclerosis. By looking at the studies that are concerned with the study of the vivo and tissue culture found that physiological LDL receptors do not use to clear Gly-LDL [8] accordingly, non-Gly-LDL has a quicker catabolic rate than Gly-LDL. It is worth mentioning that recognition by LDL receptors affected by a conformational change in the binding site due to epitopes in glycation of LDL Apo B100.

Hence, we can say there is a possibility to use scavenger receptors to clear Gly-LDL on endothelial cells and macrophages [9]; this leads to the formation of foam cells and other effects in the walls of the arteries, which in turn leads to atherosclerosis. Fig. 1, is in addition to the occurrence of glycoxidation due to modifications accrue between glycation and oxidation; it leads to generate free radicals [10]. In this study, we found that it is not necessary to increase the level of Gly-LDL in patients with diabetes compared to non-diabetic subjects because they use statin medications, and in this way, the statin lowering medications contributed to lowering the level of Gly-LDL and its use can be studied and developed for that purpose.

METHODS

Thirty-one cases of Type-2 diabetic patients (the duration of diabetes from 5 to 15 years), and 31 non-diabetic subjects recruited in this study with age range.

Both groups show an insignificant difference when matched by age, sex, and body mass index (BMI).

• Twenty-five patients diabetic taking metformin for hyperglycemia while four patients are taking metformin with insulin, but two patients are taking sitagliptin (dipeptidyl peptidase 4) for the treatment of hyperglycemia
• Twenty-eight diabetic patients are taking atorvastatin (Lipitor®), while three patients are taking simvastatin (Zocor®) as an anti-hyperlipidemia agent
• Twenty-four of the non-diabetic hypertensive subjects when taking atorvastatin (Lipitor®)
• Three subjects are taking simvastatin (Zocor®), as an anti-hyperlipidemia agent.

All patients sampled after an overnight fast with no medication (metformin, atorvastatin, simvastatin) taken within 24 h. All samples
reported to the Laboratory of Clinical Biochemistry at the Center for Strategic Diabetes Research, King Saud University, Riyadh, Saudi Arabia. The Institutional Review Board of King Saud University approved the research protocol.

The samples selected as per the inclusion and exclusion criteria; informed consent had been obtained from the subjects. A venipuncture had withdrawn 10–15 ml of fasting (12–14 h.) blood sample. A 3–5 ml of the blood withdrawn into an ethylenediaminetetraacetic acid vacutainer; the sample stored in a cold container then centrifuged for 15 min – at ×1000 g at 2–8°C – within 30 min of collection.

After 15 min, 10 ml of the remaining blood sample was separated and stored at low temperature (−20°C) for analysis within 30 of the collection of lipid profile, fasting blood sugar (FBS), glycated hemoglobin (HbA1c), and C-reactive protein (CRP) measurements.

FBS levels, HbA1c, and lipid profiles (total cholesterol [TC], LDL, HDL, and triglycerides [TG]) measured using an accent – 200 instrument, which is an open system fully automatic clinical biochemistry analyzer with user-defined profiles and calculation chemistries, manufactured by Biotek®. Sandwich enzyme-linked immunosorbent assay kits for Gly-LDL, supplied by the measuring principles depend on absorbance photometry and turbidimetry, it is resolution 0.0001 absorbance.

Using parametric and non-parametric statistics, diabetic and non-diabetic hyperlipidemic compared by analyzing the results of blood samples (lipidemic and glycemic status as standard deviation [SD]). Spearman’s rank correlation coefficient used to calculate linear regression analyses between the levels of Gly-LDL, and the data maintained as mean±SD. Statistical analysis carried out with the SPSS 18 tool. The comparison of parameters in the study groups was made by the Student’s t-test, while Pearson’s correlation coefficient determined the correlation. p<0.05 indicated statistical significance.

RESULTS

In the comparison of essential parameters, this includes diabetic patients and non-diabetic hyperlipidemic patients’ data. These data include age, BMI, Gly-LDL, LDL, HDL, TC, TG, VLDL, TG/HDL, CRP, HbA1c, and FBS. The groups of the study sample were not the same age and BMI was somewhat similar in each group. In the hyperlipidemic patients, the levels of HDL, TC, TG/HDL, CRP, Gly-LDL, and LDL were higher. On the other hand, the levels of HbA1c, VLDL, and TG were higher in diabetic subjects. The levels of HDL, LDL, and Gly-LDL were higher in the non-diabetic hyperlipidemic patient than in diabetic patients where (p=0.037), (p=0.018), and (p=0.044) respectively. The correlation between levels of Gly-LDL and parameters were used in two groups to illustrate the correlation between the concentration of Gly-LDL and the concentration of other parameters. We concluded that there is a positive correlation between Gly-LDL and HbA1c, TC, FBS, TG, LDL, VLDL, and TG/HDL, a significant correlation only noticed with LDL (p=0.035); however, a negative correlation observed with HDL and CRP in the diabetic group. In the non-diabetic hyperlipidemic group, Gly-LDL correlated negatively with HDL (p=0.048) and positively with other measured parameters, in this group Gly-LDL only significantly correlated with HDL (p=0.048), TG (p=0.035), and VLDL (p=0.031).

DISCUSSION

The presence of hyperlipidemia has a severe role in increasing the chances of developing coronary heart disease (CHD) [12-20]. Furthermore, there are physical and biochemical factors that increase the chances of developing CHD in people with DM than others, namely, lipoproteins, where lipoproteins differ in those with diabetes than those without diabetes. This difference is due to high blood sugar, changes in electrical charge, variable Apo B receptor binding, lipid synthesis changes, reduced effectiveness in suppressing intracellular cholesterol synthesis, and reduced absorption of ApoB containing lipoproteins [21-23]. All of these lead to a higher risk of CHD through
atherosclerosis due to the accumulation of lipoprotein in vivo [21,23]. The presence of the Gly-LDL increases the production of superoxide anions in macrophages, and the synthesis of prostaglandins in cell types, increasing the risk of atherosclerosis [23]. Due to these different processes, LDL is efficiently uptake by macrophages [22,23]. All of these leading to enhance the platelet agreeability and accelerating the formation of a foam cell [21,23]. In the theoretical view, we find that an increase in blood lipid levels, which in turn enhances the uptake of these lipids through macrophages in hyperlipidemia patients due to high rates of lipoprotein glycation. Therefore, the formation of foam cells should be promoted in the early stages of atherosclerosis. If we can quantify the modified lipid particles, we should identify an index of the joint atherogenicity of glycemia and lipidemia and thus devise other effective methods for identifying subjects. The result was that the level of Gly-LDL was higher in people with hyperlipidemia, more and the non-diabetic than in the diabetic ones. There is a positive correlation between Gly-LDL and HbA1c, TC, FBS, TG, LDL, VLDL, and TG/HDL; a significant correlation only noticed with LDL (p=0.035*); however, a negative correlation was observed with HDL and CRP in the diabetic group in Table 1. Furthermore, positive correlations with glucose, Hba1C, TC, TG, VLDL, and LDL, significant correlation only with TG and VLDL (p=0.035*) and (p=0.031*), and a negative one with HDL, TG/HDL, and CRP only significant with HDL (p=0.048*) in the non-diabetic hyperlipidemic group.

In diabetes, the Gly-LDL level may be higher if the levels of LDL are raised [22]; besides, the level of Gly-LDL is an essential modification of atherosclerosis of LDL. Much research needs to find an explanation for the negative results of clinical trials that seek to show a reduced risk of cardiovascular disease while improving blood sugar control [23], compared to that reduced LDL with statin treatment. The results of this study showed significantly (p=0.035) higher mean±SD serum level of Gly-LDL in the non-diabetic hyperlipidemic group (5.03±1.69 µmol/ml) as compared with the mean±SD serum level of Gly-LDL in the diabetic group (4.09±1.76 µmol/ml) (Table 2). This high level of Gly-LDL with low levels of LDL in the diabetic group is in agreement with the study of Tames et al. [24]. Furthermore, the results showed significantly (p=0.018) higher mean±SD serum level of LDL in the non-diabetic hyperlipidemic group (13.1±5.22±5.22 mg/dL) as compared with the mean±SD serum level of LDL in their diabetic patients (11.17.2±3.65 mg/dL) in Table 2, represent significantly (p=0.04) higher mean±SD serum level of TC in the non-diabetic hyperlipidemic group (185.3±30.72 mg/dL) as compared with the mean±SD serum level of TC in their diabetic patients (169.67±29.91 mg/dL). Younis et al. showed that in non-diabetic people, small dense LDL (SD-LDL) is more heavily glycosylated than other ApoB-containing lipoproteins; this can be due to increased surface exposure to lysine residues from ApoB in smaller LDL molecules (SD-LDL) [10]. Through the concentration of SD-LDL, we can determine the total plasma concentration of Gly-LDL strongly; this may be the reason for the high levels of Gly-LDL in diabetic patients who do not receive statin treatment [25]. Diabetic patients had a higher proportion of their plasma ApoB in SD-LDL than either multiple sclerosis (MS) or DM patients with a statin [26].

In DM patients, the rate of Gly-LDL in SD-LDL was higher than in large bayonet LDL; this may increase the risk of increased cardiovascular disease in MS and persisting risk in statin-treated patients [27].

Non-diabetic, normolipidemic people and those with frank diabetes appear to have an intermediate phenotype, MS; this emphasizes the correlation between plasma Gly-LDL concentration and CHD risk [10]. Michele et al. showed that the correlation between SD-LDL and plasma Gly-LDL was stronger than that with Hba1C in both diabetics and MS. While in other research, Gly-ApoB did not show any correlation with glycomic indices [24].

According to Lee et al., it is possible to find a correlation between glyemia and concentration of SD-LDL, where the concentration of SD-LDL considered on indices of glycemia in non-statin-treated Type-2 diabetes [28-30]. Although the level of LDL in both groups was within acceptable ranges, the high indication could be in the modified type of LDL, in a way that the SD-LDL will be higher, as confirmed in our study by the TG/HDL ratio. Data of the research illustrated insignificantly lower (p=0.484) mean±SD serum level of TG/HDL ratio in diabetics (3.1±1.75) as compared with the mean±SD serum level of TG/HDL in the non-diabetic hyperlipidemic group (3.5±2.37). Research data showed insignificantly lower (p=0.21) mean±SD serum level of HDL in diabetic patients (42.5±1.85 mg/dL) as compared with the mean±SD serum level of HDL in non-diabetic (hyperlipidemic) group (45.42±7.95 mg/dL). Table 2. Under the condition of hyperglycemia, the glycation of HDL can affect the activity of paraoxonase, where the presence of glycation of HDL can reduce it. Paraoxonase activity is an HDL-associated ester hydrolase, which is very important to prevent LDL oxidation. In a

### Table 1: Correlation between levels of Gly-LDL and parameters in the study

| Parameters          | Glycated LDL VS | Diabetic group | Non-diabetic | p-value |
|---------------------|----------------|----------------|--------------|---------|
| LDL (mg/dL)         | 0.035*         | 0.431          |              |         |
| HDL (mg/dL)         | 0.945 (−ve correlation) | 0.048 (−ve correlation) |              |         |
| CRP (mg/L)          | 0.406 (−ve correlation) | 0.955 (−ve correlation) |              |         |
| VLDL (mg/dL)        | 0.648          | 0.031*         |              |         |
| TG (mg/dL)          | 0.075          | 0.579          |              |         |
| HbA1c (%)           | 0.377          | 0.383          |              |         |
| FBS (mg/dL)         | 0.257          | 0.214          |              |         |

N: Number of samples=31, *p=0.05. Glycated low-density lipoprotein, LDL: Low-density lipoprotein, HDL: High-density lipoprotein, TC: Total cholesterol, TG: Triglyceride, VLDL: Very low-density lipoprotein, TG/HDL: Triglyceride/high-density lipoprotein, CRP: C-reactive protein, HbA1c: Glycated hemoglobin, FBS: Fasting blood sugar

### Table 2: Comparison of basic parameters

| Parameters          | Diabetic | Non-diabetic | p-value | Normal range |
|---------------------|----------|--------------|---------|--------------|
| Age (year)          | 49±6.9   | 46±8.9       | 0.17    | --           |
| BMI (kg/m²)         | 31.8±6.9 | 36.5±6.2     | 0.47    | --           |
| Glycated LDL (µmol/ml) | 4.09±1.7 | 5.03±1.6     | 0.037*  | <2.5         |
| LDL (mg/dl)         | 111.7±31.6 | 131.5±32.5 | 0.018*  | 100–130      |
| HDL (mg/dl)         | 42.5±6.2  | 45.4±9.7     | 0.21    | 40–60        |
| TC (mg/dl)          | 169.6±29.01 | 185.3±30.7 | 0.044*  | <200         |
| TG (mg/dl)          | 144.1±77.8 | 129.3±61.9 | 0.414   | <150         |
| VLDL (mg/dl)        | 28.4±15.7 | 25.8±12.3    | 0.468   | 12–23        |
| TG/HDL ratio        | 3.1±1.7   | 3.5±2.3      | 0.484   | <3           |
| CRP (mg/L)          | 0.39±0.47 | 0.59±0.62    | 0.32    | 0.1–0.3      |
| HbA1c (%)           | 9.0±0.018 | 9.0±0.018    | 0.000*  | <6           |
| FBS (mg/dL)         | 177±20.9 | 95±22.0      | 0.000*  | 70–100       |

Significant if the *p<0.05, SD: Standard deviation, N: Number of sample=31, HbA1c: Glycated hemoglobin, BMI: Body mass index, FBS: Fasting blood sugar, TC: Total cholesterol, LDL: Low-density lipoprotein, VLDL: Very low-density lipoprotein, HDL: High-density lipoprotein, TG: Triglycerides, CRP: C-reactive protein
previous study [30], it found that a 65% decrease in the activity of paraoxonase after observation of the presence of glycation of HDL in the sample. Furthermore, the reduction in glycated HDL and paraoxonase levels prevents monocyte adhesion to aortic endothelial cells. These are the first events with which the development of atherosclerosis begins [8]. HDL acts as a scavenger; it was observed within low levels in diabetic and non-diabetic groups, as shown in Tables 1 and 2, which demonstrates that there is an insignificant correlation between Gly-LDL and HDL levels in the diabetic group, while a significant correlation in the non-diabetic (hyperlipidemic) group observed.

CONCLUSION

- For non-diabetic (hyperlipidemic) patients, the levels of serum Gly-LDL increased. On the other hand, the levels of serum Gly-LDL reduced in diabetic patients who took a statin. We conclude that in all hyperlipidemic patients, the Gly-LDL occurs regardless of their status of glycemic
- The apparent correlation between LDL and Gly-LDL, it can be used as an indicator of controlling the glycemic in diabetic patients
- Measuring rates of Gly-LDL helps to know more about the possibility of a person infection of coronary heart disease.

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AUTHORS’ CONTRIBUTIONS

Dr. Abdulrahman Al-Bazzaz – contributed to the selection of the topic, research concept development, and guided the research process and manuscript preparation. Dr. Omar A. Al-Ani – conducted the study, participated in data processing, conducted the study, and participated in data collection and manuscript writing.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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