Glycosylated Haemoglobin (HbA₁c) - A Marker of Circulating Lipids in Type 2 Diabetic Patients

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ABSTRACT

Background: Diabetic patients with concomitant dyslipidemia are often soft targets for cardiovascular disease and deaths. An early intervention to normalize circulating lipids has been shown to reduce cardiovascular morbidity and mortality. Glycosylated hemoglobin (HbA₁c) is routinely used as a marker to indicate long-term glycemic control.

Aim: Our aim was to test whether HbA₁c can serve as a marker of circulating lipids among Type 2 diabetic patients.

Methods: The sera of 130 Type 2 diabetic patients was analyzed for fasting blood sugar (FBS), HbA₁c and lipid profile consisting of total cholesterol (TC), triglycerides (TG), High-density Lipoprotein (HDL) cholesterol and LDL cholesterol. We divided the subjects based on their glycemic index into three groups; HbA₁c< 6% as good, HbA₁c=6% - <9% as poor and HbA₁c>9% as worst glycemic control. One–way analysis of variance (ANOVA) and post-hoc Dunnett’s multiple comparison tests was used to examine the significance levels for various biochemical parameters in age-categorized groups.

Results: The mean ± SD levels of HbA₁c was significantly higher in females (8.598 ± 2.284 %) compared to males (7.323±2.18 %). Older patients had HbA₁c, FBS and lipid profile levels similar to younger ones. HbA₁c showed direct and significant correlations with cholesterol, TG and LDL. Univariate analysis showed that HbA₁c was a good predictor of circulating lipid levels.

Conclusion: The study indicates the usefulness of HbA₁c as a marker for lipid profile for screening of diabetic patients at high risk of developing cardiovascular diseases.

INTRODUCTION

Population surveys conducted over two decades in India reported a 9-fold increase of coronary artery disease (CAD) in urban centres. This increase is mainly associated with increase in the prevalence of lipid and glucose abnormalities [1]. Indians are known to have relatively lower levels of lipids and lipoproteins, raised TG and low HDL Cholesterol and presence of metabolic syndrome explaining more than half of the excess burden of CAD [2]. Diabetes mellitus is emerging as a global endemic both in developing and developed countries. It is characterized by metabolic abnormalities and long-term micro and macro vascular complications. There is a high risk of CAD in people with type 2 diabetes. Individuals with coexisting diabetes and metabolic syndrome have a high prevalence of CAD [3].

HbA₁c is commonly used as a marker of long term glycemic status. Elevated HbA₁c has also been regarded as an independent risk factor for CAD in subjects with or without diabetes. Thus elevated HbA₁c has been proposed as an independent risk factor for both diabetics and CAD patients [4]. Significantly increased levels of cholesterol and lipids are seen in type 2 diabetic patients with CAD as compared to diabetic patients without CAD [5]. It has been observed that there is a direct correlation between HbA₁c and the severity of CAD in diabetic patients [6]. The American Diabetes Association (ADA) estimated that the risk of diabetes related mortality increased 25% for each 1% increase in HbA₁c. It has also been estimated that each percentage point increase in HbA₁c corresponds to a 35% increase in the risk of macrovascular complication and an 18% increase in risk of myocardial infarction [7].

A strong correlation has been shown between lipid profile and CAD. The Framingham study has demonstrated a linear increase in CAD risk with increment of TC level from 180 mg upward. The study established that individuals with HDL cholesterol less than 35 mg/dl have 8 times increase in CAD incidence than those with HDL cholesterol more than 65 mg/dl [8]. The Lipid Research clinics Coronary Primary Prevention Trial concluded that a 1% fall in the TC reduced the CAD risk by 2% [9]. Helsinki heart study concluded that a mean 12% rise in HDL cholesterol and an 11% fall in LDL cholesterol were both correlated with a 34% decline in CAD [10].

Very few studies have shown a positive correlation between glycemic control and lipid profile. Dyslipidemia, frequently occurring in type 2 diabetes patients, might play a critical role in accelerated macrovascular atherosclerotic disease formation and may contribute significantly to the excess risk of CAD in type 2 diabetes patients [11]. Early therapeutic interventions, aiming to stabilize blood glucose levels along with reduction in TG and LDL and to increase HDL, significantly reduce cardiovascular events and mortality in patients with type 2 diabetes [12].

So control of blood glucose level may improve the lipid profile and thus reduce the risk of CAD in type 2 diabetic patients. We aimed to compare lipid profiles between uncontrolled (HbA1c<9%), moderately controlled (6%<HbA1c<9%) and controlled (HbA1c<6%) diabetic subjects to explore the association of glycemic status with CAD risk factor.

MATERIALS AND METHODS

This is a retrospective study comprising of a total of 130 type 2 diabetic patients who visited the M S Ramaiah Medical College and Teaching Hospitals, Bangalore, Karnataka, India. Ethical Clearance was obtained from the Institutional ethical committee for human research to conduct the study. The sample consisted of 94 males and 36 females. The age mean ± standard deviation of male and female subjects was 53.52 ± 11.68 years (Range 22 - 85 years) and 51.53 ±10.85 years (Range 22-77 years), respectively. All the patients were categorized into three age groups: <40 years (18 patients), 41– 60 years (85 patients), > 60 (27 patients).

Venous blood samples from all the subjects were collected in serum
separater tubes after overnight fasting. The venepuncture was done in the cubital vein. Tourniquet was used but was released just before sampling to avoid artificial increase in the concentration of serum lipids. Serum was separated within 2 hours of collection to prevent artificial changes in concentration of HDL. The blood was centrifuged at 5,000 rpm for 10 minutes. The supernatant clean serum was then pipetted out using dry piston pipettes with disposable tips and stored in dry thin walled vials at -20°C until further analysis. Care was taken to exclude the hemolysed samples. The sera were analyzed for HbA1c, FBS, TC, TG and HDL using an auto analyzer (Roche Modular P-800, Germany). The level of LDL cholesterol was determined using the Friedewald formula: LDL = (cholesterol—TG)/ (2.2 HDL) [13].

The impact of glycemic control on various parameters was evaluated by categorizing all the patients into three categories on the basis of HbA1c levels; < 6% good glycemic control, 6–9% poor glycemic control and 9% worse glycemic control. The selection of these cutoff values of HbA1c was based on earlier studies [14].

### STATISTICAL ANALYSIS

The data were analyzed by SPSS version 10. Pearson’s correlation test was performed to examine various correlations. Independent samples Student’s t-test was used to compare the means of different parameters between males and females. One-way analysis of variance (ANOVA) and post-hoc Dunnett’s multiple comparison tests was used to examine the significance levels for various biochemical parameters in age-categorized groups. Univariate analysis was performed to evaluate the effects of gender, age and glycemic control on serum lipid profile. p-value of ≤0.05 was considered as statistically significant.

### RESULTS

Our study results showed that female diabetic patients had poorer glycemic control with significant higher HbA1c (p=0.004) level when compared to males. The FBS levels were slightly higher in females as compared to male, but it was not statistically significant. Also the TC and HDL were significantly higher in females. The levels of TGs were lower and LDL was higher in females [Table/Fig-1]. There was a significant increase in HbA1c in the 41-60 years age group. Also an inverse relationship was noticed between age and glycemic control among females [Table/Fig-2]. Older patients had lower FBS, cholesterol, TGs and LDL levels though not statistically significant in comparison with the other two groups [Table/Fig-2]. Diabetic patients with poor and worse glycemic control had significantly higher levels of FBS (F = 28.387, p <0.001), TGs (F = 11.705, p < 0.001), TC (F = 11.881, p< 0.001), LDL (F = 6.301, p < 0.001) and lower levels of HDL (F = 1.555, p= 0.215) as compared to patients with good glycemic control (HbA1c ≤ 6%). Serum HDL decreases with poor glycemic control among males unlike females. However, no significant difference between HbA1c (F = 1.55, p = 0.215) as well as HDL among the patients of different age groups [Table/Fig-3,4 and 5a-f].

The results of univariate analysis addressed the impact of patient gender, age and HbA1c in influencing serum lipids [Table/Fig-4]. Patients’ gender was significantly associated with lipid parameters in decreasing magnitude in the following manner: HDL (F = 13.02), cholesterol (F = 5.04) and TGs (F = 0.811). HbA1c appeared to be a good predictor of cholesterol (F = 11.88), followed by TGs (F = 11.70), LDL (F = 6.301) and HDL (F = 1.56).

### DISCUSSION

Approximately 50-75% of deaths in patients with diabetes are attributable to cardiovascular diseases [15]. Elevated HbA1c has been regarded as an independent risk factor for CAD in subjects with or without diabetes [16]. The Diabetes Complications and Control Trial (DCCT) established HbA1c levels <7% appropriate for reducing the risk of vascular complications and also as the gold standard of glycemic control [15]. Each 1% reduction in HbA1c was associated with reduction in risk of 21% for any end point related to diabetes, 21% for deaths related to diabetes, 14% for myocardial infarction, and 37% for micro vascular complications. Thus reduction in HbA1c is associated with reduction in diabetes related risk complication [15]. Type 2 diabetic patients are at a much higher risk of cardiovascular diseases than the non-diabetic. Thus the risk of cardiovascular events in diabetics can be improved by improving the glycemic control [17].

Significant dyslipidemia has been encountered among type 2 diabetic subjects. Very few investigators have reported significant correlations between HbA1c and lipid profiles [18] and suggested the importance of glycemic control in normalizing dyslipidemia [19,20]. Also many studies have shown the beneficial effects of reducing lipids on cardiovascular system [21,22]. In our study we found that HbA1c has direct and significant correlations with FBS, TC, TG and LDL similar to many other reports [19,20]. Diabetic patients with poor and worse glycemic control had

### Table/Fig-1:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Gender of patients</th>
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<tbody>
<tr>
<td></td>
<td>Male (n=94)</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>7.32±1.7193</td>
</tr>
<tr>
<td>FBS (mg/dL)</td>
<td>142.51±52.8370</td>
</tr>
<tr>
<td>Cholesterol(mg/dL)</td>
<td>166.10±32.8454</td>
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<tr>
<td>TGs (mg/dL)</td>
<td>152.88±100.6199</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>40.49±7.9651</td>
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<tr>
<td>LDL (mg/dL)</td>
<td>96.86±27.3643</td>
</tr>
</tbody>
</table>

**Table/Fig-2:** Serum biochemistry categorized by patient’s gender

**Table/Fig-3:** Serum biochemistry categorized by patient’s glycemic control (HbA1c)

**Table/Fig-4:** Univariate analysis of variance model to evaluate the effects of gender, age, HbA1c and their interaction on serum lipid profile

**Table/Fig-5:** Percentage of patients with different parameters
Qualitative changes include size difference in lipid parameters, non-enzymatic glycosylation of LDL and susceptibility of LDL cholesterol to form peroxides, thus increasing risk of atherosclerosis and cardiovascular complications among diabetic patients [33]. Hyperglycaemia increases complications in diabetes mellitus by generating reactive oxygen species, resulting in oxidative stress. Increased lipid peroxidation causes crosslink formation between single molecules of amino acids and LDL particles. In metabolically poorly controlled diabetic patients, glycation of LDL increases with hyperglycemia [34]. This elevated level of LDL is explained by decreased catabolism of LDL, decreased activity of cholesterol ester transfer protein and lipoprotein lipase activity [21,34]. It has been suggested that non-enzymatic glycosylation of the LDL particle itself result in its increased incorporation in the arterial wall [35]. Further glycosylation of lysine groups on apolipoprotein B causes inhibition of the ability of LDL to interact with the LDL receptor. This in turn inhibits the ability of LDL to be metabolized by the LDL receptor pathway. Thus plasma LDL levels are high and atherosclerosis occurs very early in life [35]. Because of its critical importance in atherogenesis, LDL cholesterol is a focus of current guidelines for determination of the risk of cardiovascular diseases. The above discussion clearly indicates the clinical significance of various lipid parameters in predisposing diabetic patients to cardiovascular complications. Significant correlations between HbA1c and all lipid parameters and a linear relationship between HbA1c and dyslipidemia point towards the usefulness of HbA1c for screening diabetic patients at high risk of developing CAD.

LIMITATIONS

Even though our sample comprised only patients with type 2 diabetes, a population with well known high risk for CAD, other important variables were not considered in the model. Further this is a retrospective study with a small number of patients. Thus a prospective study with a large number of patients is further warranted to clearly suggest that HbA1c can be used as marker of circulating lipids. We have also not mentioned the VLDL results in this study as we did not get the required sample of VLDL tests during the study.
to statistically analyze them [38] as there have been studies which have proven that VLDL is the fundamental cause of atherosclerosis in diabetes. We have not tested the effect of glycemic control on various lipid ratios as study of these ratios have proven to be useful as markers for insulin resistance and CVD risk in T2D patients [37].

CONCLUSION

The findings of the study ensures HbaA1c predicts serum lipid profile. It provides valuable supplementary information about the extent of circulating lipids besides its primary role in monitoring long-term glycemic control. Thus, dual biomarker capacity of HbaA1c (glycemic control as well as lipid profile indicator) may be utilized for screening high-risk diabetic patients for timely intervention with lipid lowering drugs and thus preventing adverse cardiovascular events.

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REFERENCES

[33] Prabhavathi K et al., Glycosylated Haemoglobin (HbA1c) - A Marker of Circulating Lipids

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