INTRODUCTION

Prevalence of diabetes is increasing in an alarming rate worldwide. A total of 72.1 million diabetic cases have been identified in 2013 of which 65.1 million were in India [1]. Epidemiologic studies suggest that the risk of developing dyslipidemia during lifetime is said to be increased with diabetes mellitus.

It is predicted that by 2030 diabetes mellitus may afflict up to 79.4 million individuals in India, while China (42.3 million) and the United States (30.3 million) will also see significant increases in those affected by the disease [2,3]. At present, India faces an uncertain future in relation to the potential burden that diabetes may impose on the country [4]. Many factors influence affect and prevalence of disease throughout a country, and identification of those factors is necessary to facilitate change when facing health challenges. Genetic factors coupled with environmental influences such as obesity-associated with rising living standards, steady urban migration, and lifestyle changes contribute to the multifactorial etiology of diabetes in India [5-7].

Dyslipidemia is one of the common disorders which are seen in most of the diabetes patients. Insulin resistance (IR) is a metabolic disorder (IR) is a metabolic disorder independently associated with cardiovascular (CV) disease [8-10]. IR is associated with aging and a cluster of important cardiometabolic risk factors (dyslipidemia, arterial hypertension, hyperglycemia, and obesity) and is believed to be the common shared pathophysiological disturbance [11-14]. Thus, accurate and early prediction and detection of IR are very important in clinical practice so as to identify patients at high risk for CV disorders.

Apolipoprotein A1 and Apolipoprotein B are the two major apolipoproteins involved in lipid transport and in the processes causing atherosclerosis and its complications [15]. ApoB100 is the main structural apolipoprotein of low-density lipoproteins (LDL) and there is only one molecule of ApoB100 per LDL particle [16]. ApoA-I reflects the antiatherogenic potential in high-density lipoprotein (HDL) particles; the higher the value, the better the protection against CV risk [17]. Therefore, in this study, we analyzed the apolipoprotein A1 and apolipoprotein B in diabetic and non-diabetic individuals and assessed the association between IR and apolipoprotein B/apolipoprotein A-I ratio in diabetic and non-diabetic individuals.

METHODS

Study population

The study population included 416 subjects individuals of which 197 were non-diabetic and remaining 219 were non-diabetic and served as control subjects. Body mass index was calculated. Fasting plasma glucose, insulin, glycated hemoglobin levels, total cholesterol, triglyceride and high-density lipoprotein, ApoA-1, and ApoB-100 were measured using commercially available kits. Statistical analysis was performed with SPSS for Windows 16.0. Significance was defined as p<0.05.

Results:

Apolipoprotein A-1 levels were lower in the diabetic group whereas apolipoprotein B-100 levels, apolipoprotein ratios were higher in the diabetic group. ApoB100 and apolipoprotein ratio showed a positive correlation with IR.

Conclusions:

The study results indicate that apolipoprotein B100/ApoA-1 ratio can act as a strong biomarker for IR.

Keywords: Apolipoproteins, Insulin resistance, Diabetes, Dyslipidemia.
on spectrophotometer (Nycocard READER). Total cholesterol (TC), triglyceride (TG), and HDL were measured using commercially available kits (Liquichek® AGAPE).

LDL cholesterol (LDL-C) was estimated indirectly using the Friedewald formula. ApoB and ApoA1 levels were measured using commercially available kits (Agappe).

Statistical analysis
Statistical analysis was performed with SPSS for Windows 16.0. Parametric data are presented as mean±standard deviation. For categorical values, frequency counts, and percentages were applied. For group comparisons of means, t-test was applied and significance was defined as a p<0.05.

RESULTS
The results of the study cover a total of 416 subjects of which, 197 (47.3%) were diabetic and 219 (52.6%) were non-diabetic. Of which non-diabetic group includes 23% male and 29% female, and the diabetic group includes of 28.21% male and 20% female.

Age, BMI, fasting blood sugar (FBS), HbA1c, insulin, and HOMA-IR
Table 1 represents the mean age, height, weight, BMI, FBS, HbA1c, plasma insulin, and HOMA-IR values of both non-diabetic and diabetic group. FBS, HbA1c, insulin, and HOMA-IR showed a statistically significant difference between non-diabetic and diabetic individuals (p<0.05) (Tables 1-3).

Correlation between apolipoproteins, lipid ratios, and HOMA-IR in non-diabetic and diabetic group
In non-diabetic subjects, LDL/HDL ratio showed a positive correlation with HOMA-IR and negative correlation with ApoB100, whereas a negative correlation was observed between ApoA1/HDL, ApoB/LDL, and HOMA-IR values of both non-diabetic and diabetic group. FBS, HbA1c, insulin, and HOMA-IR showed a statistically significant difference between non-diabetic and diabetic individuals (p<0.05) (Tables 1-3).

Table 1: Represents the mean age, height, weight, body mass index in diabetic, and non-diabetic group

| Variables       | Non-diabetic group | Diabetic group | p-value |
|-----------------|--------------------|----------------|---------|
|                 | Mean±standard deviation | Mean±standard deviation |         |
| Age (year)      | 48.6±10.94         | 53.1±10.40     | <0.05   |
| Height (cm)     | 156.5±10.54        | 157.6±10.06    | NS      |
| Weight (kg)     | 60.0±12.06         | 62.3±12.76     | NS      |
| Body mass index (kg/m²) | 24.3±3.66     | 24.8±5.04      | NS      |

Table 2: Serum fasting glucose, insulin, and HOMA-IR in diabetic and non-diabetic group

| Variables       | Non-diabetic group | Diabetic group | p-value |
|-----------------|--------------------|----------------|---------|
|                 | Mean±standard deviation | Mean±standard deviation |         |
| FBS (mg/dl)    | 103.3±24.65        | 182.6±83.17    | <0.05   |
| HbA1c (%)      | 4.55±0.81          | 6.9±2.20       | <0.05   |
| Insulin (pmol/ml) | 27.2±4.22     | 40.2±4.22      | <0.05   |
| HOMA-IR         | 0.87±0.75          | 2.51±1.48      | <0.05   |

Table 3: Lipid profile and apolipoproteins of study subjects in diabetic and non-diabetic group

| Variables       | Non-diabetic group | Diabetic group | p-value |
|-----------------|--------------------|----------------|---------|
|                 | Mean±standard deviation | Mean±standard deviation |         |
| TC              | 180.59±64.64       | 206.5±66.26    | <0.05   |
| TG              | 175.02±91.20       | 206.4±134.78   | <0.05   |
| HDL             | 42.77±15.93        | 43.5±13.64     | NS      |
| LDL             | 102.85±49.44       | 121.38±60.39   | <0.05   |
| VLDL            | 35.00±18.24        | 41.2±26.96     | <0.05   |
| ApoA1           | 129.38±22.2        | 125.40±5.08    | <0.05   |
| ApoB100         | 76.52±1.99         | 86.20±6.23     | <0.05   |
| ApoB100/A-1     | 0.59±0.03          | 0.69±0.06      | NS      |
| ApoA1/HDL       | 2.11±0.05          | 2.01±0.73      | NS      |
| ApoB100/LDL     | 2.17±7.46          | 3.95±6.62      | <0.05   |
| LDL/HDL         | 2.93±4.22          | 3.16±2.07      | NS      |

Table 3: Lipid profile and apolipoproteins of study subjects in diabetic and non-diabetic group

p=0.05 or less was be considered statistically significant. FBS: Fasting blood sugar. HbA1c: Glycated hemoglobin, HOMA-IR: Homeostasis model assessment of insulin resistance.

In diabetic subjects, ApoB100/ApoA1 ratio also showed a positive correlation with HOMA-IR. ApoB100 showed a positive correlation with HOMA IR (Table 5).

Distribution of dyslipidemia based on patient characteristics
Total recruited diabetic (197) subjects with and without dyslipidemia have been characterized based on age, sex, control of diabetes, and obesity (Tables 6 and 7).

Dyslipidemia, apolipoproteins, and HOMA-IR in diabetic group
Individual apolipoprotein levels (ApoA1 and Apo-B100) and apolipoprotein ratios were compared in the diabetic group between dyslipidemic and non-dyslipidemic subjects. Statistically significant difference was found between dyslipidemic and non-dyslipidemic subjects with regard to ApoB100 and ApoB100/ApoA1 ratio. HOMA-IR did not show a statistically significant difference (Table 8).

DISCUSSION
In diabetic cases with metabolic abnormality, disturbances in the production and clearance of plasma lipoproteins are commonly found [18]. Diabetic dyslipidemia generally comprises postprandial lipoprotein, high TG, reduced HDL-C, and low or relatively normal LDL-C [19,18], and the development of dyslipidemia may, therefore, be a signal of future diabetes onset. Debate still exists on the nature and extent of the association between conventional lipid measures and incident Type 2 diabetes [20].

This study is a representative of dyslipidemia and apolipoprotein ratios in diabetic and non-diabetic individuals. In the present study, we found that cholesterol, TG, LDL, HDL, and very LDL (VLDL) elevated in the diabetic group compared to the non-diabetic group.

Increased levels of TC in diabetic group compared to a non-diabetic group, maybe due to an increase in the plasma concentration of VLDL and LDL, which may be due to an increase in the hepatic production of VLDL or decreased removal of VLDL and LDL from the circulation.

On studying the deranged individual lipid parameters among Type 2 diabetic subjects with dyslipidemia, the most prevalent lipid abnormality was high LDL (68%) followed by high TG (63%). An increase in the LDL in diabetic patients may be attributed to insulin. Insulin increases the number of LDL receptor, so chronic insulin deficiency might be associated with a diminished level of LDL receptor [20]. This causes an increase in LDL particles and results in the increase in LDL-C value in diabetes mellitus. Higher levels of TG may
concentration in all Asian subgroups, whether residing in India or elsewhere, is lower than Caucasians [20]. Mixed dyslipidemia was the most common dyslipidemia pattern observed in our study. The ApoB number indicates the total number of atherogenic particles; the higher the number, the higher the CV risk [21]. According to Barkas et al. [22], Apo-B represents an ideal marker for the management of dyslipidemia in individuals with diabetes.

High level of total blood cholesterol, particularly in the form of LDL-C, has been recognized for over three decades as a major risk factor for developing coronary heart disease [23]. However, recent research has shown that LDL-C is not the only lipoprotein species involved in atherogenesis. Elevated levels of intermediate-density lipoprotein and HDL-C: High-density lipoprotein, LDL: Low-density lipoprotein, HOMA-IR: Homeostasis model assessment of insulin resistance

Table 4: Correlation between apolipoproteins, lipid ratios, and HOMA-IR in non-diabetic group

| Status      | ApoA-1/HDL | ApoB/LDL | ApoB100/ApoA-1 | ApoA-1 | ApoB100 | HOMA-IR |
|------------|------------|----------|----------------|--------|---------|---------|
| Non-diabetic |            |          |                |        |         |         |
| LDL/HDL    | -0.227**   | 0.656**  | -0.013         | -0.168**| 0.004** | 0.004** |
| ApoA1/HDL  | 0.000      | 0.000    | 0.258          | 0.826  | 0.004   | 0.004   |
| ApoB/LDL   | -0.079     | 0.065    | -0.012         | 0.129   | -0.052**| 0.004   |
| Apo B100/ApoA1 | 0.913 | 0.013    | 0.844          | 0.294   | 0.027   | 0.037   |

**p=0.05 or less was be considered statistically significant. HDL: High-density lipoprotein, LDL: Low-density lipoprotein, HOMA-IR: Homeostasis model assessment of insulin resistance

Table 5: Correlation between apolipoprotein and lipid ratio in diabetic group

| Status      | ApoA1/HDL | ApoB/LDL | ApoB100/ApoA1 | ApoA1 | ApoB100 | HOMA-IR |
|------------|-----------|----------|---------------|-------|---------|---------|
| Diabetic   |           |          |               |       |         |         |
| LDL/HDL    | -0.651**  | 0.713**  | -0.103        | -0.109| 0.116   |         |
| ApoA1/HDL  | 0.000     | 0.000    | 0.193         | 0.165 |         |         |
| ApoB/LDL   | -0.247**  | 0.251**  | 0.009         | 0.317**| 0.041   |         |
| Apo B100/ApoA1 | 0.003 | 0.002    | 0.913         | 0.000 | 0.626   |         |
| ApoA1      | -0.043    | 0.017    | -0.044        | 0.126 |         |         |
| Apo B100   | -0.605**  | 0.814**  | 0.312**       | 0.000 | 0.000   |         |

**p=0.05 or less was be considered statistically significant. HDL: High-density lipoprotein, LDL: Low-density lipoprotein, HOMA-IR: Homeostasis model assessment of insulin resistance

Table 6: Patient characteristics and prevalence of dyslipidemia in diabetic group

| Characteristic | No. of patients (n=197) | % | Patients with dyslipidemia (n=129) | % |
|----------------|-------------------------|---|------------------------------------|---|
| Age            |                         |   |                                    |   |
| 30–45          | 57                      | 28.93 | 23                               | 17.82 |
| 45–60          | 140                     | 71.06 | 106                              | 82.17 |
| Sex            |                         |   |                                    |   |
| Male           | 107                     | 54.31 | 77                               | 59.68 |
| Female         | 90                      | 45.68 | 52                               | 40.31 |
| Control of DM  |                         |   |                                    |   |
| Controlled     | 79                      | 40.10 | 35                               | 27.03 |
| Uncontrolled   | 117                     | 59.39 | 94                               | 72.97 |
| Obesity        |                         |   |                                    |   |
| BMI>23         | 120                     | 60.91 | 97                               | 75.19 |
| Non obese (BMI<23) | 76                     | 38.57 | 32                               | 24.80 |

Table 7: Pattern of dyslipidemia in diabetic group

| Variables | Diabetic subjects with dyslipidemia n (%) |
|-----------|------------------------------------------|
| Mixed dyslipidemia | High TG, high LDL-C, and low HDL-C | 57 (44.2) |
| Combined dyslipidemia | High TG and low HDL-C | 12 (92) |
| High TG and high LDL-C | 15 (10.8) |
| High HDL-C, and low HDL-C | 10 (7.8) |
| Isolated single parameter dyslipidemia | High TG | 11 (87.4) |
| High LDL | 15 (12.16) |
| Low HDL | 10 (14.3) |

TG: Triglyceride, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, HDL-C: High-density lipoprotein cholesterol concentration in all Asian subgroups, whether residing in India or elsewhere, is lower than Caucasians [20]. Mixed dyslipidemia was the most common dyslipidemia pattern observed in our study.

The ApoB number indicates the total number of atherogenic particles; the higher the number, the higher the CV risk [21]. According to Barkas et al. [22], Apo-B represents an ideal marker for the management of dyslipidemia in individuals with diabetes.

High level of total blood cholesterol, particularly in the form of LDL-C, has been recognized for over three decades as a major risk factor for developing coronary heart disease [23]. However, recent research has shown that LDL-C is not the only lipoprotein species involved in atherogenesis. Elevated levels of intermediate-density lipoprotein and

Percentage in each group is from total patient in that group. BMI: Body mass index

also be due to insulin deficiency in hyperglycemia and mobilization of fatty acids from adipose tissue.

The current study is not in accordance with few previous studies, which showed lower levels of HDL in diabetic patients. The study done by Bodhe et al. [18], on Indians showed that HDL-C levels varied with glycemic control. As in our study, all patients with diabetes showed higher HDL level when compared to non-diabetic individuals but were statistically insignificant. The reason for insignificant result as also suggested by Garg et al., 2016 [1], appears that average HDL
VLDL are also associated with increased CV risk. All these potentially atherogenic lipoproteins contain one ApoB molecule and therefore, the total ApoB value indicates the total number of potentially atherogenic lipoproteins [24,25]. The association of ApoB with incident Type 2 diabetes has been proven with improved risk prediction compared to LDL-C or HDL-C [24]. Thus, ApoB has been found to be a better predictor of risk than LDL-C, VLDL, and chylomicrons.

ApoA-I reflects the antiatherogenic potential in HDL particles; the higher the value, the better the protection against CV risk [26], but the ApoA-I was lower in diabetic group, this was not in accordance to the previous studies by Onat et al., 2010 [26] who reported that high ApoA1 levels independently predicted incident Type 2 diabetes among a sample of Turkish participants.

In diabetic subjects, ApoB100/ApoA1 ratio also showed a positive correlation with HOMA-IR, suggesting that as apolipoprotein ratio increases, IR also increases. ApoB100 also showed a positive correlation with HOMA-IR.

The ApoB/ApoA-I ratio indicates the balance between atherogenic and antiatherogenic particles; the higher the value, the higher the CV risk, the ApoB/ApoA ratio is strongly associated with IR [25]. However, only a few studies have shown the associations between apolipoprotein levels and the risk of diabetes. Hwang et al. [27] indicated that the ApoB/ApoA1 ratio is an effective predictor of T2DM in the Korean population. The ApoB/LDL-C ratio has been associated with T2DM in a population-based study of Turkish adults 14 and ApoB in the Aboriginal Canadian population [28].

CONCLUSIONS
Based on our study results, it is clear that aggressive dyslipidemia management is the need of the hour in diabetic individuals. In diabetic individuals, the overall control rate of dyslipidemia is low. The pattern of lipid profile of the study may, therefore, be a significant risk factor for the increased rate of CV problem in diabetes than normal individuals. ApoA1/ApoB-100 was highly associated with IR in both non-diabetic and diabetic groups. Further study is needed to determine the role of ApoA1/HDL-C in the development of diabetes, as susceptible individuals are increasingly considered as candidates for appropriate interventions.

ACKNOWLEDGMENTS
This project is supported by Nitte University grant (NUFR1) by Nitte (Deemed to be University) with grant no.NUFR1/2016/16-04.

AUTHORS’ CONTRIBUTIONS
Both authors have contributed equally to planning, execution, data analysis, preparation, and editing of the manuscript.

CONFLICTS OF INTEREST
We declare that there are no conflicts of interest.

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