Original Research Article

Dyslipidemia in Prediabetes Population: A Retrospective Study of 91780 Cases

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ABSTRACT

Dyslipidemia over time leads to atherogenesis in both diabetics as well as prediabetics. The study aims to assess the correlation if any between the blood glucose levels [HbA1c] and the lipid cholesterol levels among the prediabetes group.

Method: A total of 91780 cases were reviewed across India from the same group of laboratory across 7 years from Jan 2015 to May 2022. The lipid profiles and glycated haemoglobin [HbA1c] were tested in all of them. The separation and quantification of hemoglobin A1c (HbA1c) in EDTA whole blood was done on the Tosoh HLC-723 G8 glycohemoglobin analyzer, an ion exchange HPLC instrument. Analysis of reports of lipid profile which includes triglycerides, total cholesterol, HDL cholesterol LDL cholesterol was done using enzymatic colorimetric test method on Roche analyzer. The prediabetes group was classified based on the HbA1c levels and the lipid profile levels were analysed among them.

Results: The analysis showed percentage of prediabetes cases was 40.36%. Age group of >60 years had the maximum prediabetes cases (79.65%), whereas female group had slightly higher proportion prediabetics then males (62.09% VS 61.05%). Prediabetes group showed a significant association with borderline HDL (OR: 1.14, p value <0.0001), major risk HDL (OR: 1.33, p value <0.0001), desirable non HDL cholesterol (OR: 1.31, p value <0.0001), borderline high non HDL cholesterol (OR: 1.54 p value <0.0001), high non HDL cholesterol (OR: 1.60, p value <0.0001) and very high non HDL cholesterol (OR: 1.65, p value <0.0001). We also observed a significant association of borderline high triglycerides (OR: 1.22, p value <0.0001) and high triglycerides (OR: 1.89, p value <0.0001). High VLDL was also associated with pre diabetics (OR: 1.31, p value <0.0001).

Conclusion: The study showed that most of the lipid profile parameters were higher in the prediabetes group as compared to the healthy non-diabetic group. The HDL levels were shown to be lower among the prediabetics as compared to the non-diabetics. A mandatory lipid profile screening among prediabetes patients will prevent further cardiovascular risk among them.

Keywords: Diabetes, prediabetes, glycated haemoglobin [HbA1c], dyslipidaemia, high density lipoprotein [HDL], Low density lipoprotein [LDL], cholesterol

INTRODUCTION

Diabetes is a chronic, metabolic disease characterized by elevated levels of blood glucose (or blood sugar), which leads over time to serious damage to the heart, blood vessels, eyes, kidneys and nerves. The most common is type 2 diabetes mellitus, usually in adults, which occurs when the body becomes resistant to insulin or doesn't make enough insulin [1].

As per the **American Diabetic Association (ADA)** based on the blood marker values diabetes is defined as any of the following abnormalities:

- Haemoglobin A1c 6.5% or higher

- Fasting plasma glucose 126 mg/dL or higher
- A 2-hr glucose of 200 mg/dL or higher after a 75-g oral glucose load (oral glucose tolerance test).

Prediabetes refers to an intermediate stage of dysglycemia along the spectrum from normoglycemia to diabetes [7]. Prediabetes is identified by laboratory measurement of fasting blood glucose (FBG), glycosylated haemoglobin (HbA1C), or 2-h post load blood glucose (2hBG) [7]. According to the American Diabetes Association (ADA) and WHO, prediabetes can be classified as:

- HbA1c 5.7 to 6.4%
- Fasting plasma glucose 100 mg/dL to 125 mg/dL
- A 2-hr glucose of 140 mg/dL to 199 mg/dL after a 75-g oral glucose load (oral glucose tolerance test).

Those with HbA1c below 5.6% are considered as non-diabetics. The term prediabetes is used to identify those individuals who are at risk for future diabetes. It is also associated with a high burden of cardio metabolic risk factors and is associated with poor outcomes [8]. In recent years, there has been an increased awareness about the prevalence of prediabetes, a high-risk state for developing diabetes. The end-organ target damage in prediabetes and the associated adverse outcome is indistinguishable to that of diabetes.

Dyslipidemia is the imbalance of lipids (fats) such as cholesterol, low-density lipoprotein cholesterol, (LDL-C), triglycerides, and high-density lipoprotein (HDL). This condition can result from diet, tobacco exposure, lifestyle, stress or genetic factors and can lead to cardiovascular disease with severe complications. [10]

Dyslipidemia and cardiovascular disease are major contributors to morbidity and mortality in diabetes mellitus [2].

Cardiovascular risk factors such as insulin resistance, glucose intolerance, and abnormal lipid profile are the key contributors of atherogenesis in diabetes. Dyslipidemia or abnormal lipid profile levels plays a crucial role in atherogenesis, in both prediabetes and diabetes, characterized by elevated triacylglycerol (TG), reduced high-density lipoprotein (HDL), and predominant small density-low density lipoprotein (LDL). Dyslipidemia and its correlation with HbA1c and fasting blood glucose (FBG) has been studied extensively in diabetic patients, but very few studies have been done so far among prediabetes individuals on their lipid profile pattern and its possible correlation with FBG or HbA1c (9).

Aim of the study- To assess the association of prediabetes with lipid metabolism disorders to understand whether lipid profile screening should be proposed for individuals with prediabetes.

MATERIALS & METHODS
A total of 91780 cases were reviewed across India in the same chain of laboratories for 7yrs (from Jan 2015 to May 2022). Cases who had done both lipid profile and HbA1c were only included in this study. The age and gender of the individuals were obtained from the lab records. Individuals falling in the diabetes group were excluded for the correlation analysis.

Inclusion criteria were:
- Cases grouped as prediabetes with HbA1c in the range of 5.7 to 6.4%.
- Cases who had done both HbA1c as well as lipid profiles testing.
- Criteria for dyslipidaemia was as per "The National Cholesterol Education Programme (NCEP) Adult Treatment Panel III Reporting guidelines"

Exclusion criteria were:

- Cases grouped as diabetes with HbA1c more than 6.4% (as per the WHO criterion)
- Cases who had done only HbA1c or lipid profile test.

Sample collection and Analysis:

Blood samples were obtained for biochemical tests.

Analysis of HbA1c:

Tosoh G8 HPLC instrument was used for separation of HbA1c.

- ➢ **Principle of Tosoh G8 separation:**
  The HLC -723G8 is a fully automated high performance liquid chromatography instrument -reagent system that rapidly and precisely separates hemoglobin found naturally in blood. Charged hemoglobin and other hemoglobin components are eluted at varying times depending on the net charge of the molecule in relation to gradient of increasing ion strength passed through a non- porous cationic exchange column (negatively charged beads).

  In the HbA1c variant analysis mode predetermined windows are set in the software to detect the presence of hemoglobin A1a, A1b, F, I-A1c and s-A1c and A0.

  Also H-V0, H-V1 and H-V2 are used as a first indication that there is a haemoglobinopathy present. Calibration is for the quantitation of the stable A1c fraction. All other windows are for presumptive identification for various haemoglobin.

- ➢ **Reference Range**
  American Diabetes Association (ADA) and WHO guidelines classifies individuals based on HbA1c as:
- Non-diabetic: <= 5.6
- Pre-diabetic: 5.7-6.4
- Diabetic: => 6.5

Analysis of lipid profile:

Analysis of reports of lipid profile which includes triglycerides, total cholesterol, HDL cholesterol was done using enzymatic colorimetric test method on Roche analyzer. VLDL cholesterol, CHOL/HDL ratio, LDL/HDL ratio, LDL Cholesterol, serum Non-HDL Cholesterol are calculated parameters.

Reference ranges for lipid profile were defined as per

The National Cholesterol Education Programme (NCEP) Adult Treatment Panel III Reporting guidelines:

Parameters	Interpretation (in mg/dl)
LDL Cholesterol	Optimal: < 100 Near Optimal / Above Optimal: 100-129 Borderline High: 130-159 High: 160-189 Very High: >= 189
Triglycerides level	Normal: < 150 Borderline High: 150-199 High: 200-499 Very High: >= 500
Cholesterol-Total	Desirable – Up to 200 Borderline high - 200-240 High - Above 240
HDL Cholesterol	Major risk factor for heart disease: < 40 Negative risk factor for heart disease: >= 60
Non HDL Cholesterol	Optimal: < 130 Desirable: 130-159 Borderline high: 159-189 High: 189-220 Very High: >= 220
LDL Cholesterol	Optimal: < 100 Near Optimal: 100-129 Borderline high: 130-159 High: 160-189 Very High: >= 190
VLDL Cholesterol	6-38
LDL/HDL RATIO	2.5-3.5
CHOL/HDL RATIO	3.5-5

STATISTICAL ANALYSIS

Data recording was done in MS Excel. Continuous variables are reported as Mean$\pm$ Standard Deviation (SD). Discrete variables are summarized in terms of frequencies and

percentages. Shapiro-Wilk test was used to determine whether data sets differed from a normal distribution.

For comparison of continuous variables between two groups unpaired t test was used and for comparison of categorical variables Chi square test has been used.

Pearson's correlation coefficient was used to analyze the relation of HbA1C (Glycated Haemoglobin) with cholesterol, HDL, CHOL/HDL ratio, LDL, LDL/HDL ratio, Non HDL, triglycerides and VLDL

To determine the independent association of lipid profiles with prediabetes, a multiple Logistic Regression analysis was performed by classifying prediabetics as 1 and non-diabetics as 0 (dependent variable) and results were reported as ODD Ratio (OR) and p values.

All statistical analysis was performed using "R Studio version 1.4.1103". A two-tailed p value of <0.05 was considered to be statistically significant.

RESULT

The prevalence of Diabetics was observed to be 34.52% and Prediabetics was 40.36%. For further analysis, diabetics cases were excluded from the study.

Table 1: Prevalence data

HbA1c	Frequency	Percentage
Non-Diabetic	23062	25.13%
Pre Diabetic	37038	40.36%
Diabetic	31680	34.52%

Age range were divide into 4 parts with maximum cases falling in age range of 31 – 45 years (32.78%) followed by 46 – 60 years (30.11%) and >60 years (24.99%). Female cases were slightly more than male (55.47% VS 44.53%).

Table 2: Demographic prevalence

	Frequency	Percentage
Age Group		
18 – 30	7284	12.12%
31 - 45	19701	32.78%
46 – 60	18096	30.11%
>60	15019	24.99%
Gender		
Female	33338	55.47%
Male	26762	44.53%

The results obtained using categorical Chi-square test showed that:

Age and gender were statistically significant associated with HbA1c. Age group of >60 years had the maximum pre diabetics cases (79.65%), whereas female group had slightly higher proportion prediabetics then males (62.09% VS 61.05%). All parameters of Lipid profile showed statistically significant association.

Table 3: Association of non-diabetics and prediabetics with demographics and lipid profiles.

	HbA1C Glycated Haemoglobin				
	Non-Diabetic		Pre Diabetic		
	N	%	N	%	p value
Age Group (in Years)					
18 – 30	5033	69.10%	2251	30.90%	<0.0001
31 - 45	9644	48.95%	10057	51.05%	
46 – 60	5328	29.44%	12768	70.56%	
>60	3057	20.35%	11962	79.65%	
Gender					
Female	12638	37.91%	20700	62.09%	0.0090
Male	10424	38.95%	16338	61.05%	
Cholesterol Total					
Desirable	16332	40.07%	24427	59.93%	<0.0001
Borderline high	5215	35.94%	9294	64.06%	
High	1515	31.35%	3317	68.65%	
HDL Cholesterol					
Negative Risk	3726	43.49%	4841	56.51%	<0.0001
Borderline Risk	13661	38.81%	21540	61.19%	
Major Risk	5675	34.75%	10657	65.25%	
CHOL/HDL Ratio					
Low	9140	42.77%	12228	57.23%	<0.0001
Normal	9945	37.21%	16780	62.79%	
High	3977	33.12%	8030	66.88%	

Table 3 To Be continued…					
LDL Cholesterol					
Optimal	9255	39.82%	13988	60.18%	<0.0001
Near Optimal	7874	40.22%	11704	59.78%	
Borderline High	4288	35.39%	7830	64.61%	
High	1298	32.18%	2735	67.82%	
Very High	347	30.76%	781	69.24%	
LDL/HDL Ratio					
Low	13474	40.88%	19482	59.12%	<0.0001
Normal	6716	36.60%	11635	63.40%	
High	2872	32.66%	5921	67.34%	
Non HDL Cholesterol					
Optimal	11465	41.65%	16064	58.35%	<0.0001
Desirable	6487	38.02%	10576	61.98%	
Borderline High	3472	33.98%	6745	66.02%	
High	1262	31.28%	2773	68.72%	
Very High	376	29.94%	880	70.06%	
Triglycerides Level					
Normal	17941	40.57%	26284	59.43%	<0.0001
Borderline High	3215	31.88%	6869	68.12%	
High	1906	32.91%	3885	67.09%	
VLDL Cholesterol					
Normal	20959	39.31%	32361	60.69%	<0.0001
High	2103	31.02%	4677	68.98%	

The cholesterol, triglycerides, LDL, and VLDL levels were found to be higher in the prediabetes group as compared to the healthy non-diabetic group. The HDL levels were shown to be lower among the prediabetics as compared to the non-diabetics. The non HDL levels, CHOL/HDL ratio, and LDL/HDL ratio was higher in the prediabetes group as compared to the healthy non-diabetes group.

Table 4: Comparison of HbA1c with lipid profile.

	HbA1C Glycated Haemoglobin		
	Non-Diabetic	Pre Diabetic	p value
Cholesterol	180.05+37.59	184.43+40.76	<0.0001
HDL	48.25+12.72	46.68+11.71	<0.0001
CHOL/HDL ratio	3.96+1.25	4.14+1.23	<0.0001
LDL	109.78+32.74	112.11+35.97	<0.0001
LDL/HDL	2.43+1.01	2.54+1.01	<0.0001
Non HDL	132.79+36.87	137.75+39.70	<0.0001
Triglycerides	115.05+52.19	128.17+51.78	<0.0001
VLDL	22.74+10.62	25.57+10.32	<0.0001

A weak positive correlation was seen in Triglycerides (r= 0.1390, p value= <0.001) and VLDL (r= 0.1507, p value= <0.001).

Table 5: Correlation of HbA1c (prediabetes and non-diabetes) with lipid profile.

	Correlation Coefficient (r)	95% CI of r	p value
Cholesterol	0.04064	0.03266 to 0.04862	<0.0001
HDL	-0.06947	-0.07742 to -0.06151	<0.0001
CHOL/HDL ratio	0.07205	0.06409 to 0.08000	<0.0001
LDL	0.02865	0.02066 to 0.03663	<0.0001
LDL/HDL	0.05037	0.04239 to 0.05834	<0.0001
Non HDL	0.06330	0.05533 to 0.07126	<0.0001
Triglycerides	0.1390	0.1311 to 0.1468	<0.0001
VLDL	0.1507	0.1429 to 0.1585	<0.0001

Multiple logistic regression analysis was used to identify independent association of lipid profiles with prediabetes.
Pre Diabetic showed a significant association with borderline HDL (OR: 1.14, p value <0.0001), major risk HDL (OR: 1.33, p value <0.0001), desirable non HDL cholesterol (OR: 1.31, p value <0.0001), borderline high non HDL cholesterol (OR: 1.54 p value <0.0001), high non HDL cholesterol (OR: 1.60, p value <0.0001) and very high non HDL cholesterol (OR: 1.65, p

value <0.0001). We also observed a significant association of borderline high triglycerides (OR: 1.22, p value <0.0001) and high triglycerides (OR: 1.89, p value <0.0001). High VLDL was also associated with pre diabetics (OR: 1.31, p value <0.0001).

Table 6: Multiple logistic regression analysis.

Parameters	Multiple logistic regression			
	Variable	p value	OR	95% CI OR
Cholesterol Total	Normal	Ref		
	Borderline	0.7871		
	High	0.0624		
HDL Cholesterol	Negative	Ref		
	Borderline	<0.0001	1.1425	1.0806 to 1.2080
	Major Risk	<0.0001	1.3307	1.2319 to 1.4374
CHOL/HDL Ratio	Normal	Ref		
	High	0.0223	0.9290	0.8795 to 0.9813
	Low	0.0084	0.9080	0.8354 to 0.9870
LDL Cholesterol	Optimal	Ref		
	Near Optimal	<0.0001	0.8015	0.7565 to 0.8493
	Borderline high	0.0001	0.8073	0.7313 to 0.8913
	High	0.0068	0.8017	0.6831 to 0.9409
	Very High	0.0116	0.8037	0.6119 to 1.0555
LDL/HDL Ratio	Normal	Ref		
	High	0.4763		
	Low	0.0588		
Non HDL Cholesterol	Optimal	Ref		
	Desirable	<0.0001	1.3134	1.2322 to 1.4000
	Borderline high	<0.0001	1.5375	1.3764 to 1.7175
	High	<0.0001	1.6063	1.3509 to 1.9100
	Very High	0.0004	1.6540	1.2493 to 2.1898
Triglycerides level	Normal	Ref		
	Borderline high	<0.0001	1.2204	1.1588 to 1.2853
	High	0.0283	1.8966	1.8133 to 1.9885
VLDL Cholesterol	Normal	Ref		
	High	<0.0001	1.3100	1.2046 to 1.4245

DISCUSSION

The incidence of diabetes and its impact on the cardiovascular health is a fast increasing global health concern. Most of the diabetic conditions and its associated complications may be preventable if detected in the early or the prediabetes stages. There are some studies that have recently been done among the prediabetics which further support this.

A retrospective study that tracked lipid profile and atherogenic indices among prediabetics of Andaman Nicobar Islands, noted that all the lipid profile components except HDL were significantly increased and HDL was significantly decreased in prediabetics [11]

A cross sectional study done by Rao et al at the Shree Krishna Sevashrama Hospital, Bangalore, Karnataka, India from April 2017 to January 2019 on the Assessment of lipid profile and demographic data in South Indian prediabetics had noted statistically significant changes only in the HDL component of lipid profile and the significant presence of abdominal obesity. BMI did not show any significant association with prediabetes. [9]

Similarly, in a study of myocardial performance index in prediabetes and its correlation with other cardiovascular risk factors, assessment of lipid profile, BMI, Waist Hip Ratio (WHR), it was noted that BMI, WHR, TC, TG, LDL, VLDL were all significantly higher and HDL was significantly lower in prediabetes subjects [12]

The current study also showed similar results with levels of LDL, triglycerides and total cholesterol increasing as the HbA1c levels increase and the HDL levels reducing inversely with respect to HbA1c.

Analysis of the data obtained by Korea National Health and Nutrition examination survey from the study of their prediabetics

had shown significantly higher levels of Total TC, TG, LDL, TC to HDL-C ratio, TG to HDL-C ratio, LDL-C to HDL-C ratio, and significantly increased urinary albumin excretion in prediabetic women [13].

Our current study focussed only on the lipid profile levels. The BMI, WHR, urine albumin levels were not studied.

In a study done by Chakraborty M, Singh P, Dsouza JMP, Pethusamy K, Thatkar PV, lipid profile and blood glucose were analyzed in fasting and postprandial blood samples of fifty-one diabetics and thirty-two prediabetics which reflected postprandial TG and the TG/HDLc lipid abnormalities than the corresponding fasting variables in diabetes and prediabetes [5].

The study done by Al Amri et all on 613 individuals showed that even though high LDL-C is associated with an increased probability of prediabetes, a recommendation for universal screening of dyslipidaemia patients requires further cohort studies [14].

In the study of young Saudi prediabetics which assessed their lipid profile, total antioxidant status (TAS), demographic, and lifestyle data showed statistically significant changes in all the components of the lipid profile and the presence of significantly increased BMI in their study. There was a positive correlation between HbA1c and TC, TG, LDLC, BMI, and central obesity while a negative correlation was reported between HbA1c and HDL Cholesterol and TAS [15].

The other novel cardiac markers like hs-CRP, homocysteine, lipoproteins and Lp-PLAC may also be considered for comparison. But, since our study was mainly done based on lipid profiles these parameters were not analysed.

All these studies further supported our current analysis.

Our study showed that the cholesterol, triglycerides, LDL, and VLDL levels were found to be higher in the prediabetes group as compared to the healthy non-diabetic group. The HDL levels were shown to be lower among the prediabetics as compared to the non-diabetics. The non HDL levels, CHOL/HDL ratio, and LDL/HDL ratio was higher in the prediabetes group as compared to the healthy non-diabetes group.

CONCLUSION

Dyslipidemia or abnormal lipid levels lead to atherogenesis that is thickening or clogging of the arteries which lead to impaired blood supply to the vital organs, mainly the heart. Chronic or long standing type 2 diabetes has been known to affect the lipid levels which in turn impact the arterial supply. The significance of similar effects among the prediabetes group is an area that requires further research. Our study establishes that there is a significant correlation between the cholesterol, LDL, HDL levels and HbA1c levels. Our aim should be towards reducing the LDL (bad cholesterol) and triglyceride levels and increasing the HDL (good cholesterol) levels in prediabetes group itself to prevent complications going ahead.

Thus regular lipid profile testing should be made mandatory even in the prediabetes group to prevent them from going into the diabetes group and reduce their risk of developing hypercholesterolemia and cardiac disorders.

Ethical Approval: Not Applicable

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