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Role of Serum Lipid and Apolipoprotein Ratios in Prediction of Diabetic Retinopathy

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Abstract: Aims: The objective of the study was to determine clinical utility of different ratios of serum lipid and apolipoprotein as predictive markers for diabetic retinopathy (DR). **Methodology:** In this case control study, study participants were divided in to four groups, normal controls, diabetic controls, non-proliferative diabetic retinopathy (NPDR) and proliferative diabetic retinopathy (PDR). Staging was done based on fundoscopy by an ophthalmologist. A fasting venous blood was assayed for Lipid profile, levels of ApoA1 and ApoB and different combination of ratios for these tests were used to assess association with DR. **Results:** Independently, only ApoB levels proved to be predictive of DR, but being non-specific to DR it cannot be used as independent marker. Amongst various ratios, only ratios of apolipoproteins were significantly associated with DR. **Conclusion:** ApoB/ApoA1 ratio is the most suited ratio for screening of DR.

Keywords: Diabetic Retinopathy, Lipid ratios, Apolipoproteins, Predictive biomarkers

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Introduction

Amongst non-communicable disease (NCD), Type II Diabetes Mellitus is not only the most prevalent disease, but its global burden is increasing rapidly. Current global burden is reported to be 415 million with an expected count to be 624 million by 2040 [1]. It is recognized as an important cause of early death and disability and is one of four diseases considered to be priority NCD's by the world leaders [2]. Diabetes requires prompt diagnosis and management due to its spectrum of complications that not only cause disability but also lead to mortality.

Diabetic retinopathy (DR) is the retinal consequence of chronic progressive diabetic microvascular leakage and occlusion. It eventually occurs to some degree in all patients with diabetes mellitus. There are two types: non-proliferative (NPDR) and proliferative (PDR). NPDR is the early stage of the disease and is less severe. Blood vessels in the eye tend to leak fluid into the retina with the passage of time, which leads to blurred vision. Proliferative diabetic retinopathy (PDR) is the more advanced form of the disease. New blood vessels start to grow in the eye (neovascularization), which are fragile and can lead to formation of micro-aneurysm and hemorrhage, resulting in vision loss and scarring of the retina [3].

DR has become a major public health issue in Asian countries in recent years with significant increase in DR [4, 5]. Diabetic retinopathy ranks third in economic burden generated by complications of diabetes [6]. About 40% of diabetics develop retinopathy within 40 years of disease while 50.2% progressed to advanced retinopathy [7]. In another study prevalence of total blindness due to T2DM was reported to be 11.4% while total burden of DR was reported to be 43.1% [8] While another study suggests that 60% of diabetics develop DR within 20 years of diagnosis of DM.[9]. Similar findings have been reported in various studies in Pakistan [10-14]. A total burden of DM in Pakistan is reported to be 32% [15], while regarding prevalence of DR, different studies suggest different prevalence ranging from 21% (n=163) [16] to 33% (n=66, amongst newly diagnosed cases of DM) [14] and 42.8% (n=66) [17].

Development of screening program for early prediction of DR has become a necessity not only due to huge burden on health care system but also to decrease financial burden on patient and health care system as well as to maintain quality of life of the patient. Several biochemical markers are being studied to establish an association between DR and their presence prior to development of this complication. There is a need to study emerging and novel biomarkers which are specific to retinopathy and have potential to predict its early onset [18].

It has been reported that Apo B is present in retina in DR and Apo A1 is an indicator of increased lipids in peripheral tissues. Both the apolipoprotein have their role in pathogenesis of DR which are major contributor of DR in DM. Ratio of both the markers can be used as predictive model for DR excluding other known causes in which there is increased ApoA1 and Apo B.[19].Similarly retinol binding protein 1 and 4, interleukins, tumor necrotic factor alpha, vascular endothelial factor and neutrophil gelatinase-associated lipocalin are used as markers for DR [19-23]. Other than these biomarkers some studies including molecular studies like miro-RNA’s or genome wide sequence for early prediction of DR [24-26]. Other than genomic markers use of apolipoprotein, retinol binding proteins and fatty acid binding proteins as biomarkers are suggested in some studies to be most reliable markers for early prediction of DR. Use of these markers independently for prediction of retinopathy is not only conflicting but also has implications of healthcare finances. So, we propose use of different ratios of serum lipid fractions as well as lipoprotein fractions that are routinely done in most of the labs.

Aims and Objectives

The objective of the study was to determine clinical utility of different ratios of serum lipid and apolipoproteins predictive markers for DR.

Methods:

Participants:

The study was case control in nature, minimum sample size was calculated using OpenEpi, which estimate sample size to be 85. Keeping the estimated margin of error to be 5% and confidence level of 99.9%. The anticipated prevalence was reported in literature (for Pakistan) was found to be 2% Prevalence of DM is 9.8% while that of DR in DM is reported to be 21% [27] (study with most appropriate sampling and methodology), raking a prevalence of DR to be 2.05 in total population of Pakistan. The study participants were divided into cases and controls (35 controls and 44 cases). Both the cases and controls were further stratified into healthy control, diabetic controls (without DR) as controls and cases of NPDR and PDR. Consecutive sampling was done. Inclusion and exclusion criteria was:

<table>
<tr><th>Group</th><th>Controls</th><th>Diabetic control</th><th>Cases of NPDR</th><th>Cases of PDR</th></tr>
<tr><td>Inclusion</td><td>Disease free, adult human subjects.</td><td>Diagnosed cases of DM without DR.</td><td>Diagnosed cases of NPDR</td><td>Diagnosed cases of NDR</td></tr>
<tr><td>Exclusion</td><td colspan="4">Abnormal lipid profile or patients on lipid modifying drugs or patients with any metabolic disorder, Any ocular disorder or family history of any ocular defect (other than refractory errors), History of epilepsy</td></tr>
</table>

Sample Collection Protocol:

A fasting sample of 3cc venous blood was drawn in Gel tube, 3 cc in EDTA tube and 3cc in sodium fluoride+ Calcium oxalate tubes using BD vacutainer blood sampling method and in BD vacutainers. All the tests were performed on Roche Cobas C-501 closed system. All the samples were analyzed for HbA1C, fasting glucose, lipid profile (including total cholesterol, triglyceride (TG), HDL- cholesterol, LDL- cholesterol, Apo-A1, Apo-B100.

Diagnosis of PDR/NPDR:
Diagnosis of PDR and NPDR was wad done by consultant ophthalmologist on fundoscopy.

Data Analysis:
Data analysis was done using IBM SPSS v 23. Mean and SD will be calculated for lab results. Kriskol-Willis was applied to determine the association of the markers and the ratios within the study groups.
Ethical approval was sought from the institutional Ethical review committee.

Results:
Our study included a total 79 subjects, of which 14(17.7%) were normal control, 21 (26.6%) diabetic controls while in cases 19 (24%) were NPDR and 25 (31.6%) were PDR. There were 60 males while only 19 females in our study. Basic demographic data for the study participants according to study groups is shown in (**table 1**).

Table 1: Basic demography

	Controls		Diabetic control		Cases of NPDR		Cases of PDR	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Age	42.86	6.84	60.57	11.59	62.21	8.56	55.52	7.63
Weight(kg)	68.43	15.19	79.05	14.73	76.16	14.60	71.20	9.60
Height (meter)	1.65	0.09	1.64	0.39	1.69	0.08	1.66	0.06
BMI (kg/m^2)	24.59	3.78	25.67	7.20	27.06	6.72	25.97	4.12
DM duration (years)	--	--	5.28	3.20	8.82	6.53	12.24	5.46
DR Duration (months)	--	--	--	--	5.73	3.74	8.86	7.38

The glycemic control was found to be almost similar in the diabetic control as well as both the cases, glycemic control was assessed using HbA_{1C} levels. ApoA1 levels were found maximum in NPDR group while ApoB levels were to be highest in diabetic control, similarly Cholesterol, TG and LDL were found to be maximum in PDR group with an increasing trend, while HDL showed inverse behavior. Table 2a and 2b, (**Figure 1)**

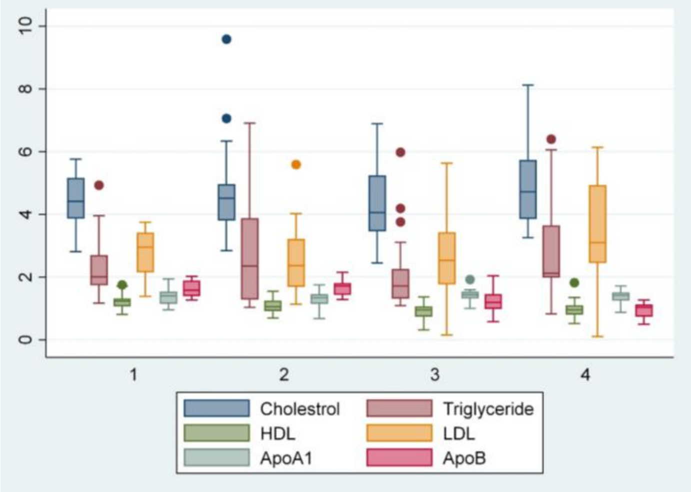

Figure 1 Comparison of lipid profile and apolipoproteins in the study groups

Different combinations of ratios of lipid profile and apolipoproteins were applied to test their clinical utility. (**Table 3**) shows comparison of these ratios.

Table 2: Mean and SD for glycemic parameters

	Controls		Diabetic control		Cases of NPDR		Cases of PDR	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Glucose (mmol/L)	5.43	.91	12.94	6.73	11.91	7.38	13.22	5.97
HbA1c (%)	5.74	.56	9.04	2.43	9.27	1.58	9.64	2.50

Table 2 (b): Lipid profile and apolipoproteins

	Controls		Diabetic control		NPDR		PDR		**p-value**
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Cholesterol (mmol/L)	4.43	0.86	4.71	1.50	4.28	1.22	4.98	1.35	0.39
Triglyceride (mmol/L)	2.35	1.07	2.76	1.59	2.21	1.25	2.84	1.55	0.517
HDL (mmol/L)	1.24	0.28	1.06	0.22	0.93	0.26	0.98	0.29	0.114
LDL (mmol/L)	2.81	0.77	2.50	1.10	2.69	1.26	3.40	1.70	0.031
Apo A1 (µmol/L)	1.35	.28	1.27	.27	1.41	.22	1.35	.24	0.341
Apo B (µmol/L)	1.62	0.26	1.68	0.26	1.19	0.35	0.95	0.23	<0.001

Table 3: Comparison of ratios of lipid profile and apolipoproteins across groups

	Controls		Diabetic control		Cases of NPDR		Cases of PDR		**p-value**
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Cholesterol: TG	2.13	0.68	2.16	1.08	17.51	66.79	7.65	26.56	0.939
Cholesterol: HDL	3.72	0.94	4.51	1.63	4.82	1.43	5.37	1.67	0.009
Cholesterol: LDL	1.64	0.24	1.97	0.36	10.88	34.65	10.18	31.81	0.01
TG: Cholesterol	0.53	0.24	0.59	0.28	0.49	0.23	0.56	0.38	0.939
TG: HDL	2.14	1.38	2.64	1.54	2.54	1.45	3.15	1.77	0.31
TG: LDL	0.94	0.56	1.16	0.62	1.20	1.57	1.63	2.54	0.51
HDL: Cholesterol	0.29	0.08	0.25	0.08	0.22	0.09	0.20	0.09	0.01
HDL: TG	0.60	0.27	0.54	0.31	0.51	0.25	0.49	0.41	0.31
HDL: LDL	0.48	0.18	0.50	0.23	0.74	1.68	0.88	2.51	0.048
LDL: Cholesterol	0.62	0.08	0.52	0.11	0.59	0.24	0.64	0.27	0.01
LDL: TG	1.29	0.48	1.13	0.58	1.37	0.66	1.41	0.74	0.51
LDL: HLD	2.33	0.71	2.44	1.20	3.09	1.51	3.71	1.99	0.048
ApoA1: ApoB	0.86	0.27	0.77	0.20	1.30	0.48	1.51	0.55	<0.001
ApoB: ApoA1	1.25	0.36	1.39	0.39	0.86	0.28	0.74	0.21	<0.001

As the data was non-uniformly distributed, Kriskol Willis test was applied between the analytes independently as well as the ratios, which showed that HDL was able to differentiate only control and PDR group, while ApoB was significantly different in control VS both retinopathy groups as well as in diabetic and retinopathy groups, at the same time there was no significant NPDR and PDR group as well as control and diabetic (without DR) inferring that ApoB can be used to in establishing presence of retinopathy but unable to differentiate either it is proliferative or non-proliferative (**table 4**). (non-significant variables not included in the table)

Table 4: Association of DR with different markers

Comparison group	Control-Diabetic Control	Control -NPDR	Control - PDR	Diabetic Control-NPDR	Diabetic - Control PDR	NPDR-PDR
HDL	0.642	0.290	0.031	1.000	1.000	1.000
ApoB	1.000	<0.001	<0.001	<0.001	0.009	0.278

Among different ratios lipid and apolipoproteins ratios only apolipoprotein ratios (both ApoB/ApoA1 and ApoA1/ApoB) were able to distinguish between all the groups except control group and diabetic control (without DR). Also, both the ratios were unable to differentiate either it is proliferative or non- proliferative, inferring that both the ratios can be used to in establishing presence of retinopathy but unable to differentiate either it is proliferative or non- proliferative (**Table 5, Fig 2**.)

Table 5: Association of different rations of with DR

Comparison group	Control-Diabetic Control	Control -NPDR	Control -PDR	Diabetic Control-NPDR	Diabetic - Control PDR	NPDR-PDR
Cholesterol: HDL	0.946	0.217	0.009	1.00	0.337	1.00
Cholesterol: LDL	0.32	1.00	1.00	0.169	0.01	1.00
HDL: Cholesterol	1.00	0.172	0.01	1.00	0.348	1.00
HDL: LDL	1.00	0.080	0.723	0.048	0.647	1.00
LDL: Cholesterol	0.32	1.00	1.00	0.169	0.01	1.00
LDL: HDL	1.00	0.080	0.048	0.723	0.647	1.00
ApoB: ApoA1	1.00	<0.001	<0.001	<0.001	0.038	1.00
ApoA1: ApoB	1.00	<0.001	<0.001	<0.001	0.029	1.00

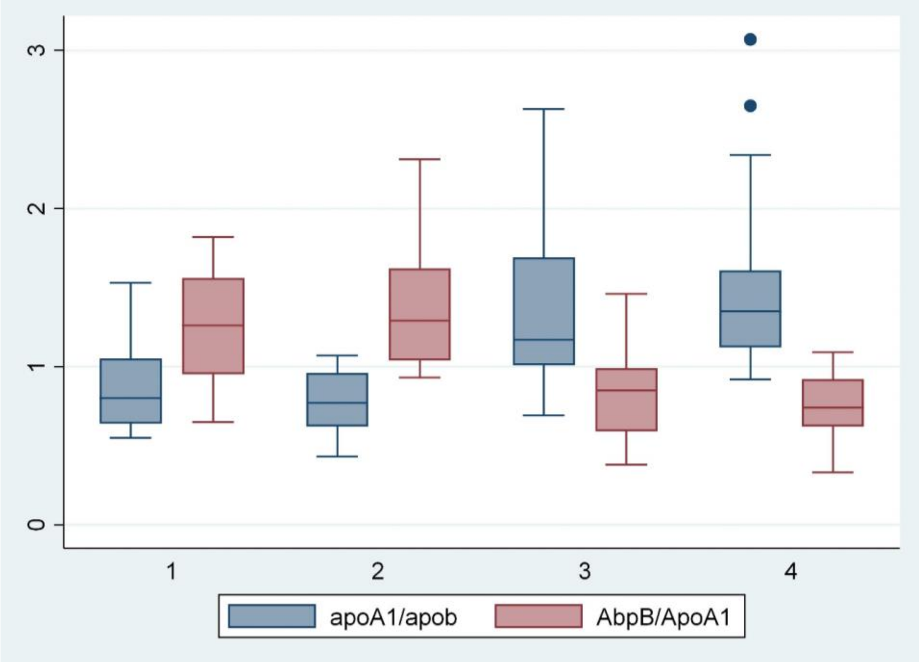

Figure 2 Comparison of apolipoproteins ratios in the study groups

Discussion:

Numerous studies have shown associations between lipid levels and macrovascular complications of diabetes milieus such as coronary artery disease. Relatively few studies have paid attention to the association of serum lipid profile with DR, and there are also some inconsistencies in these available clinical outcomes as reported to date. Our study showed that amongst lipid profile, only High-Density Lipids (HDL) showed significant association with DR, yet only able to differentiate between late-stage DR (PDR) and control group, while an inverse correlation with DR. While LDL, total cholesterol and TG were found to have non-significant association with DR. Similarly, their distribution across groups showed inconsistent relationship. Our results regarding HDL and LDL are inconsistent with previous study from India [28] a more recent report by Zhang also suggested that all the four analytes had a non-significant association with DR [29]. While Ajith reported that only ApoB/ApoA1 ratio is significant while all other parameters independently are non-significant [30]. Regarding apolipoproteins, our study suggested that There a strong association of ApoB with DR but no association of ApoA1. Literature shows controversial evidence regarding the association of these apolipoproteins Ankit *et al.*, [28] suggested both the lipoproteins as well as ApoB/ApoA1 had strong association towards DR while Bhasker [22] suggested that ApoA1 is more strongly associated with DR than ApoB, similarly we used ApoA1/ApoB ratio rather than ApoB/ApoA1 ratio that is reported to be significant by our as well as several other studies and reported it to be significant as well.

ApoB/ApoA1 ratio is still a better marker as compared to ApoA1/ApoB as so far decreased ApoB/ApoA1 ratio is reported in DR so far which is in harmony with our results [19, 28]. ApoA1 is known to have anti-inflammatory and antioxidant properties that specifically inhibits LDL oxidation. The oxidized LDL deteriorates the antiplatelet and anti-inflammatory functions of endothelium. Hence a parameter is needed that reflects the effects produced from the interaction of both these lipoproteins which seems to be crucial in the pathogenesis of microangiopathy leading to DR. For this reason, this ratio can be suggested to be useful which is not only proven by our study but also many others [8]. Only deviation found in our results was that both the ratios failed to differentiate between NPDR and PDR. This might be attributed to smaller sample size. Andina reported HDL/LDL ratio and ApoB/ApoA1 ratio to be significant [31]. ApoA1/ApoB ratio is associated with several conditions such as renal impairment, coronary events. No other study has yet reported other ratios described in our study.

Conclusion:

Our study concludes that ratios of cholesterol, triglyceride, HDL, and LDL cannot be used to predict or diagnose diabetic retinopathy, but ApoB/ApoA1 ratio can be used as a screening tool for diabetic retinopathy and selected cases can then be further be evaluated by fundoscopy thus reducing the burden on physician as well.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Ethical approval was sought from institutional ethical review committee. Ethical approval #27022019. Informed consent was taken from each individual participant.

HUMAN AND ANIMAL RIGHTS

No animals were used in this study. The study on humans was conducted in accordance with the ethical rules of the Helsinki Declaration and Good Clinical Practice.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

None.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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