ORIGINAL ARTICLE

Correlation Study of Vascular Cell Adhesion Molecule-1 and Remnant Lipoprotein Cholesterol with Diabetes Retinopathy

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ABSTRACT:

Background: The aim was to look into the role of vascular cell adhesion molecule-1 (VCAM-1) and remnant lipoprotein cholesterol (RLP-C) in patients of type 2 diabetes with retinopathy and its comparisons with the healthy non-diabetic controls.

Methods: 34 normotensive newly diagnosed Type 2 diabetic patients with retinopathy and 15 healthy normotensive non-diabetic age and sex match controls. Cases and controls were taken from 1st September 2016 to 31st June 2018. All cases undergo thorough routine investigations. The obtained information regarding the levels of fasting blood sugar (FBS), post prandial blood sugar (PPBS), Glycosylated Hemoglobin level (Hb1Ac), Lipid Profile, 24 hours urinary protein, VCAM-1 Levels, Remnant lipoprotein levels were analyzed, the statistical analysis was performed using SPSS for windows version 16.0 software. For comparing two group of mean Students't' test and for paired samples Paired 't' test was used. The p value <0.05 was considered as statistically significant.

Results: In our study majority of the patients were in age between 51 and 60 years. Male outnumbered females with ratio of 1.8:1. The mean level of FBS, PPBS, HbA1c, 24 hours urinary protein, cholesterol, triglyceride, VLDL, LDL and remnant lipoprotein cholesterol were significantly higher in patients than in controls, in the meantime the mean value of VCAM-1 and HDL were significantly lower in patient than the control.

Conclusion: VCAM-1 is not positively correlated with diabetic retinopathy. While RLP-C positively correlated with diabetic retinopathy as compared to healthy control, but its usefulness as marker for diabetic retinopathy, needs further studies with large sample size.

Key words: Vascular Cell Adhesion Molecule (VCAM), Remnant Lipoprotein Cholesterol (RLP-C), Diabetic Retinopathy,

INTRODUCTION

Diabetes mellitus is a leading cause for the cardiovascular diseases, blindness, amputations and end stage renal disease in the world.[1] The most common form of diabetes mellitus is type 2 and constitutes about 90% of all the diabetic cases.[2,3]

Diabetes mellitus (DM) is associated with a wide range of microvascular complications including diabetic retinopathy (DR), currently estimated to be the leading cause of blindness in working-aged adults.[1]The prevalence of any retinopathy in persons with diabetes is 35% while proliferative (vision threatening) retinopathy is 7%.[4]

Studies suggested that diabetic retinopathy is an inflammatory disease and that adhesion molecules may be involved in the pathogenesis of this ocular disorder.[5,6,7].The interaction between leukocytes and endothelium vessels is governed by cellular adhesion molecules of the immunoglobulin super-family, including vascular cell adhesion molecule-1(VCAM-1) expressed on endothelial cells and their receptors leukocytes function antigen-1 (LFA-1), andvery late antigen-4 (VLA-4), expressed on leukocytes.[8] Soluble VCAM-1 is released from cytokine-activated vascular endothelial cells.[9] and determination of soluble VCAM-1 may predict dysfunction of vascular endothelium, leading to diabetic retinopathy.

Elevated remnant lipoprotein cholesterol (RLP-C) levels were found to be associated with endothelial dysfunction, an early marker for atherosclerotic disease.[10] The Framingham Heart Study found that RLP-C levels was positively correlated as significant risk factor for coronary artery disease in women.[11]

Nakamura et al reported that high levels of RLP-C predict ischemic stroke in patients with metabolic syndrome and mild carotid atherosclerosis.[12]However, its contribution to development of diabetic retinopathy remains unexplored.

Present study is aimed to look into the role of vascular cell adhesion molecule-1(VCAM-1) and remnant lipoprotein cholesterol (RLP-C) in patients of type 2 diabetes with retinopathy and its comparisons with the healthy non-diabetic controls, as data of retinopathy are scanty from our country and especially from this region.

MATERIAL AND METHODS

The present study was conducted in the Department of General Medicine and endocrinology, Institute of Medical Sciences, Banaras Hindu University, Varanasi in collaboration with Department of Biochemistry in the period of month of June 2017 to July 2018.

35 patients of newly diagnosed type 2 diabetics with retinopathy of age between 20 to 65 years were selected from the Department of Medicine and Endocrinology IMS, BHU, Varanasi. 15 Age and sex matched healthy non-diabetic &Normotensive individuals were selected as the controls.

Detailed history and clinical examination (including fundoscopy by ophthalmologist) was done in all the selected cases and controls. Then the venous blood samples of about 5 ml were collected. 3 ml was taken in a clean and dry plain vial without any anticoagulant. The blood was kept to clot at room temperature. The sera were removed and stored at -20^{0}C in a sterile plain glass vial until analyzed. 2ml of blood was also taken in EDTA vial for analysis.

Before final estimation of VCAM-1 and remnant lipoprotein cholesterol level all the cases and controls underwent complete blood counts, Renal function test, Liver function test, Plasma glucose fasting and post prandial, HbA1c, Urine R/M, 24 hour urinary protein, Electrocardiogram, 2D echocardiogram (optional, clinically suspected coronary artery disease patients only).The Criteria for diagnosis of diabetes was adapted from American Diabetes Association 2017. The patient was excluded who were on multivitamins and mineral therapy, smoking, who were working in chemical / asbestos / metal factories, receivingchemotherapy and radiotherapy, with hypertension, on drugs like metformin, linagliptin and glibenclamide.

Estimation of Remnant Lipoprotein Cholesterol (RLP-C): Remnant lipoprotein cholesterol level was estimated by using ELISA method. Using Purified Human RLP-C antibody to coat Micro ElisaStripplate wells to make solid-phase antibody, then add serum and RLP-C antibody labeled with HRP, to wells, then the final complex become antibody-antigen-antibody-enzyme complex.After washing, TMB substrate solution added, TMB substrate becomes blue color under HRP enzyme action, reaction is terminated by the addition of a sulphuric acid solution and the final color change is measured spectrophotometrically at a wavelength of 450 nm. The concentration of RLP-C in the samples is then determined by comparing the optical density (O.D.) of the samples to the standard curve. The sensitivity of this kit was 5.0μmol/L.

Estimation of VCAM-1 Level:VCAM-1 level of samples was estimated with the use of RayBio® Human VCAM-1 ELISA Kit. This kit was an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of human VCAM-1 in serum, plasma, and cell culture supernatants. This assay had an antibody specific for human VCAM-1 coated on a 96well plate. Standards and samples were pipetted into the wells and VCAM-1 present in a sample bound to the wells by the immobilized antibody. Then wells were washed and biotinylated anti-human VCAM-1 antibody was added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin was pipetted to the wells. The wells were again washed, and then a TMB substrate solution was added to the wells and color develops in proportion to the amount of VCAM-1 bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color was measured at 450 nm wavelength by spectrophotometer.

Statistical Analysis: The statistical analysis was done using SPSS for Windows version 16.0 software. Descriptive statistics like mean, frequency and percentages of various parameters were calculated. For categorical variable Chi-Square test and Fischer's Exact test was used. For comparing two group of mean Students 't' test and for paired samples Paired 't' test was used. The p value <0.05 was considered as statistically significant.

OBSERVATION AND RESULTS

The present study was conducted in the Department of Medicine, Endocrinology and Biochemistry of Institute of Medical Sciences, Banaras Hindu University. The study consisted of Group 1 (Thirty-fourpatients of Normotensive newly diagnosed Type-II Diabetics with retinopathy) & Group 2 (Fifteen healthy non-diabetic and non-hypertensive) age and sex matched controls.

Mean age of group 1 is 50.15±9.88 and mean age of group 2 is 55.27±5.78. Male Female ratio is 1.6:1 and 1.1:1 for respective groups (**Table 1**).In our study, among the diabetics with retinopathy patients, 7(20.6%) were in age group of 30-40 whereas in the control group had none. 7(20. %) of diabetes with

Table 1: Characteristics of study Population

Variables	Group I (n=34)	Group II (n=15)	Total (n=49)
Age (in yrs)	50.15±9.88	55.27±5.78	
Age group			
31-40	7 (20.6%)	0 (0.0%)	7 (14.3%)
41-50	7 (20.6%)	4 (26.7%)	11 (22.4%)
51-60	17 (50.0%)	8 (53.3%)	25 (51.0%)
>60	3 (8.8%)	3 (20.0%)	6 (12.2%)
Gender distribution			
Male/Female	1.6: 1	1.1: 1	1.45:1
Male	21 (61.8%)	8 (53.3%)	29 (59.2%)
Female	13 (38.2%)	7 (46.7%)	20 (40.8%)

Table 2: Lab characteristics of the study population

Group	Mean ± SD	p-value
Fasting blood sugar		
Group I (n=34)	216.21 ± 72.75	P<0.001 (HS)
Group II (n=15)	91.87 ± 9.28	
Post Prandial Blood sugar		
Group I (n=34)	307.76 ± 97.04	P<0.001 (HS)
Group II (n=15)	129.00 ± 7.59	
HbA1c		
Group I (n=34)	9.86 ± 1.71	P<0.001 (HS)
Group II (n=15)	5.86 ± 1.71	
24 hours urinary protein		
Group I (n=34)	66.85 ± 56.55	P<0.05 (S)
Group II (n=15)	30.93 ± 18.44	

Table 3: Lipid parameters of the study population

Group	Mean ± SD	p-value
Cholesterol		
Group I (n=34)	207.76 ±14.03	<0.001 (HS)
Group II (n=15)	152.60 ±22.87	
Triglyceride		
Group I (n=34)	206.35±15.28	<0.001 (HS)
Group II (n=15)	124.40±9.62	
VLDL		
Group I (n=34)	43.32±4.42	<0.001 (HS)
Group II (n=15)	21.2±4.57	
LDL		
Group I (n=34)	104.44±13.89	<0.05 (S)
Group II (n=15)	94.07±11.09	
HDL		
Group I (n=34)	31.32±6.32	<0.001 (HS)
Group II (n=15)	41.93±5.87	

Table 4: VCAM-1 and RLP-C levels in study groups

Group	Mean ± SD	p-value
Vascular cell adhesion molecule-1 (VCAM-1)		
Group I (n=34)	13.91±7.36	<0.001 (HS)
Group II (n=15)	25.35±6.66	
Remnant-Lipoprotein (RLP-C)		
Group I (n=34)	312.86±133.60	<0.01 (HS)
Group II (n=15)	204.34±59.13	

retinopathy patients were in the age group of 41-50 year while 11(22.4%) were in controls.17(50.0%) diabetic with retinopathy patients were in the 51-60 years of age group whereas 8(53.3%)were in the control group. 3(8.8%) diabetic with retinopathy patients were in the more than 60 years of age group while 3 (20%)were in control group. The maximum number of diabetic patients with retinopathy were 17(50.0%) in the age range of 51-60 years. Also, the maximum number of healthy controls were 8 (53.3%) in the age range of 51-60 years, p=0.227 (**Table 1**).

Mean value of FBS in group is216.21 ± 72.75 and that in group 2 is 91.87 ± 9.28, so it is significantly higher in group 1 (**Table 2**). The mean value PPBS in group 1 is 307.76 ± 97.04 and that in group 2 is 129.00 ± 7.59 (**Table 2**). The mean value of HbA1c in group 1 is 9.86 ± 1.71 and that in group 2 is 5.86 ± 1.71 (**Table 2)**.The mean value of24 hours urinary protein in group 1 is66.85 ± 56.55 and that in group 2 is 30.93 ± 18.44 (**Table 2)**. The mean value of cholesterol in group 1 is 207.76 ±14.03and that in group 2 is 152.60 ±22.87 (**Table 3)**. The mean value of triglycerides in group 1 is 206.35±15.28 and that in group 2 is 124.40±9.62 (**Table 3).** The mean value of VLDL in group 1 is 43.32±4.42and that in group 2 is 21.2±4.57 (**Table 3).**The mean value of LDL in group 1 is 104.44±13.89 which is significantly higher 94.07±11.09 than in group 2 (**Table 3**). The mean value of HDL in group 1 is 31.32±6.32 which is significantly lower than in group 2 (41.93±5.87) (**Table 3**). Mean value of VCAM-1 in group 1 is 13.91±7.36 and group 2 is 25.35±6.66, significantly lower in group-1 (**Table 4)**. Mean value of RLP-C in group 1 is 312.86±133.60 and group 2 is 204.34±59.13, significantly higher in group 1. **(Table 4)**.

DISCUSSION

Diabetes mellitus is the leading cause of preventable blindness. Blindness is due to proliferative diabetic retinopathy and clinically significant macular edema. The prevalence of retinopathy in persons with diabetes is 35%.[4]Early diagnosis of this microvascular complications of diabetes is essential for the prevention of visual impairment. Sincethe development of DR depends on several parameters, that's why making itsprediction is difficult.

The objective of this study was to compare the levels of VCAM-1 and RLP-C in patients of type-2 diabetes mellitus with retinopathy as compare to nondiabetic healthy persons, to look for any significant correlation. So that these two study molecules can be used as potential biochemical marker of early diabetic retinopathy.

Studies have suggested that diabetic retinopathy is an inflammatory disease and that adhesion molecules may be involved in the pathogenesis of this ocular disorder.[5,6,7] Inflammation is initiated by activation of endothelial cells by inflammatory cytokines.[13] This activation leads to an increase in the expression of cellular adhesion molecules such as vascular cell adhesionmolecule-1 (VCAM-1) on endothelial cells. Regulation of the expression of these adhesion molecules is critical for leukocyte migration through endothelial cell junctions.[14] In diabetic patients, leukocytes adhere and accumulate in retinal vessels. Most of them leave vessels and transmigrate in the retina.[5.] The interaction between leukocytes and endothelium vessels is governed by cellular adhesion molecules of the immunoglobulin super-family, VCAM-1 expressed on vessels and their receptors, LFA-1, and VLA-4, expressed on leukocytes.[8]. The soluble form of VCAM-1 has been detected in endothelial cell culture fluids and human sera.[9] Soluble VCAM-1 is released from cytokine-activated vascular endothelial cells[9] and determination of soluble VCAM-1 may predict dysfunction of vascular endothelium or microvascular complications.

Other similar studies about role of VCAM-1 was done in 2008 on conjunctiva of diabetic and non-diabetic patients which concluded "VCAM-1 are up-regulated in the conjunctiva of diabetic patients with and without retinopathy, reflecting the inflammatory nature of thiscondition and suggesting a possible role for these mediators in the pathogenesis of diabetic retinopathy."[15]

In our study we compared soluble VCAM-1 in serum of type-2 diabetes mellitus patient with retinopathy and healthy subjects not having any disease. The mean value of VCAM-1 in diabetic patient with retinopathy was 13.91±7.36 and mean value in healthy subjects was25.35±6.66 in our study. The P value of study was P<0.001 which was highly significant.So, we found there is significantnegative correlation between VCAM -1 level with type-2 diabetic with retinopathy.

Remnant Lipoprotein Cholesterol (RLP-C)

Studies published as early as 1952 have suggested an association between hard exudates and serum lipids **(16).** According to Goliasch, high remnant lipoprotein cholesterol is more predictive of myocardial infarction than any other lipid particle. Remnant cholesterol is especially predictive of coronary artery disease in patients with normal total cholesterol **(17).**Bernelot Moens SJ et al. have demonstrated remnant lipoprotein cholesterol, contributing to cardiovascular disease risk in patients with familial dysbetalipoproteinemia**(18)** However its contribution to development of diabetic microvascular complications remains unexplored

The present study revealed significant relations between Total cholesterol, triglycerides, LDL, HDL and VLDL molecules in diabetic retinopathy patients and controls. The value of HDL is significantly lower in diabetic retinopathy patients as compared to healthy control, whereas values of LDL and VLDL were higher in retinopathy patients as compared to healthy control.The present study showed significantly high concentration of remnant lipoprotein cholesterol in diabetic retinopathy patientsas compared to healthy controls which indicate that RLP-Ccan be useful as marker of diabetic retinopathy, but confirmation of this need's further studies with larger sample size.

CONCLUSION

Previous studies suggested that diabetic retinopathy as low grade inflammatory state, we want to study relation between inflammation induced endothelial adhesion molecule VCAM-1 with diabetic retinopathy. But in our study, we not found any positive correlation between VCAM-1 and diabetic retinopathy. We also studied relation of RLP-C a marker of endothelial dysfunction, with diabetic retinopathy. In this study we found significantly higher level of RLP-C in diabetic retinopathy patients as compared to healthy nondiabetic control. This finding may indicate that RLP-C can be a potential marker of diabetic retinopathy, but confirmation of this need's further studies with larger sample size.

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