

Association of Coronary Artery Diseases with ABO and Lewis Blood Group Phenotypes at a Tertiary Care Teaching Hospital in Southern India

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ABSTRACT

Introduction: Since the discovery of blood group systems, involvement of ABO system to coronary artery disease was suggested. Epidemiological data on the association of ABO and Lewis blood group with coronary artery disease from Southern India was not available.

Aim: To assess the pattern and association of ABO and Lewis blood group phenotypes in confirmed Coronary Artery Disease (CAD) patients attending the tertiary care hospital in Southern India.

Materials and Methods: The present study was a single centric case control analytic study where 187 clinically confirmed CAD cases were compared with age and gender matched 187 healthy controls. ABO grouping and Lewis antigen typing were determined to know the association with CAD and its risk factors. Statistical analysis was done using SPSS version 20.0 and by computing categorical variables in percentage.

Results: Blood group O was the most common (41.2%) blood group in the controls followed by blood group B (33.2%), A (21.9%), AB (3.7%). The prevalence of O group in the CAD patients was almost similar to controls, but comparatively, the frequency of Non O groups showed a mild increase in the CAD patients; the frequency of AB groups in these patients was comparatively less than the controls. The prevalence of Le (a-b-) phenotype has been observed to be 32.6%. We observed that 94.1% of cases were associated with risk factors like 'Smoking', 'Hypertension', 'Diabetes mellitus', 'Dyslipidemia'.

Conclusion: The present study failed to show a significant association of ABO blood group with CAD but showed a significant association of ABO group with risk factors like hypertension, dyslipidemia and smoking. It also showed a significant association of Le (a-b-) phenotype with CAD and with risk factors like diabetes mellitus, dyslipidemia and smoking.

Keywords: ABO blood group system, Lewis negative phenotype, Ischaemic disease

INTRODUCTION

Blood group antigens of the ABO and Lewis systems are not only expressed on the Red Blood Cell (RBC) membrane but are also expressed on the surface of the platelets, the vascular endothelium, sensory neurons, and epithelium [1]. Lewis (Le) antigens are found primarily in the secretions and the plasma and are adsorbed onto the RBC membrane [2].

Studies report that blood Group A individuals are more susceptible to cardiovascular diseases [3]. The major one was the Framingham study which reported that 'A' phenotype people are more susceptible for CAD [4]. Wazirali H et al., suggested that blood group A increases the risk of CAD independent of conventional risk factors [5]. Some Indian studies also reported that the incidence of CAD in blood Group A Bengalese of North Bengal is higher compared to other blood groups [6]. A meta analysis report by Chen Z et al., comprising 17 studies showed that the risk of CAD was slightly higher in blood group A and lower in blood group O [7]. In contrast, Garg P et al., reported a significant association between CAD and blood group B [8]. Sahita P et al., from Gujarat, India, did not observe any association between blood group and CAD after excluding patients with risk factors [9].

Patients with CAD were observed to have a significant, almost 2.5 times higher prevalence of Le (a-b-) phenotype compared to controls [10]. Salomaa V et al., observed that carotid intima-media thickness was slightly higher among persons with Lewis

negative phenotype than among persons with Lewis positive phenotype [11]. Lewis (a-b-) blood group has been reported to be associated with hypercholesterolemia, diabetes mellitus and insulin resistance conditions which are considered to be high risk factors for CAD [12]. The association between the Lewis genotype with subclinical carotid atherosclerosis was studied by Cakir B et al., who reported a statistically non significant association between Lewis genotype and subclinical atherosclerosis [13].

In view of the above varying reports, we have planned to undertake the present study to assess the association of ABO and Lewis phenotypes in CAD patients attending the tertiary care hospital in Southern India.

MATERIALS AND METHODS

The present study was a single centric case-control analytical study conducted in the Department of Transfusion Medicine, Sri Venkateswara Institute of Medical Sciences, Tirupati, Andhra Pradesh, India, from February 2015 to August 2016 after obtaining approval from the Institutional Ethics Committee (IEC No: 443/09-03-2015). Inclusion criteria for the cases were patients between the age group of 20-65 years with a diagnosis of CAD on the basis of history of chest pain, electrocardiographic changes of ischaemia like ST elevation and associated risk factors like history of smoking, hypertension, diabetes mellitus and changes in the lipid profile. Only those cases who underwent percutaneous transluminal coronary angioplasty at the cardiology department of the institute and had angiographically positive result were included in the study. Exclusion

criteria were those above 65 years of age, patients with other co-morbid illness like malignancy, pregnant females, patients who were not willing to participate in the study. Age and Gender matched healthy blood donors who were eligible to donate blood as per the inclusion and exclusion criteria of Drugs and Cosmetics Rules, 1945 were taken as controls [14].

After obtaining written informed consent from each of the concerned subjects, 3 mL of blood in Ethylene Diamine Tetra Acetic acid (EDTA) anticoagulant tube was collected separately from each CAD patient as well as from blood donors. Collection of blood samples repeatedly from the same subject were avoided by personally verifying the subject's demographic profile such as full name, age, address, including hospital registration number and by collecting the blood sample in required quantity.

ABO blood grouping was performed according to department's Standard Operational Procedures (SOP). Commercially available monoclonal blood group antisera (Tulip diagnostics Pvt. Ltd., Verna, Goa, India) were used for forward grouping; for reverse grouping 5% pooled suspension of A, B and O cells were used which were prepared according to SOP.

Lewis antigen typing was done by column agglutination technology method using monoclonal anti sera Le^a, Le^b (Ortho Clinical Diagnostics, High Wycombe, UK) in a reverse diluent cassette (Ortho Clinical Diagnostics, High Wycombe, UK) as per the manufacturer's instructions.

STATISTICAL ANALYSIS

Statistical analysis was carried out using SPSS version 20.0. Descriptive Statistics for the categorical variables were performed by computing the frequencies (percentages) in each category. Comparison of categorical data between association of ABO and Lewis phenotypes in clinically confirmed CAD patients was done using chi-square test. Incidence was given in proportion with 95% Confidence Interval (CI). All statistical analysis was carried out at 5% level of significance and p-value <0.05 was considered as significant. To allow isolation of the effect of risk factors on CAD, multivariable logistic regression models were used to estimate odds ratios and CI for the association of ABO and Lewis phenotypes and other risk factors relative to CAD.

RESULTS

During the study period, 187 patients with a diagnosis of CAD were investigated to know the association of the CAD with ABO and Lewis blood group phenotypes along with age and gender matched control group of 187 healthy voluntary blood donors who attended present blood bank.

Both the CAD group and control group had 166 (89%) males and 21 (11%) females. The [Table/Fig-1] shows distribution of ABO blood groups among the CAD cases and controls. Group O was the most prevalent followed by group B in both cases and controls and there was no significant association between CAD cases and controls (p-value=0.39). The [Table/Fig-2] shows distribution of Lewis phenotypes in CAD cases and controls. Le (a-b-) was the most prevalent phenotype (61.5%) followed by Le (a-b+) (37%)

Blood groups	Cases		Control		p-value*
	Number	Percentage	Number	Percentage	
O	77	41.2%	80	42.8%	0.39
B	62	33.2%	58	31.0%	
A	41	21.9%	35	18.7%	
AB	7	3.7%	14	7.5%	
Total	187	100%	187	100%	

[Table/Fig-1]: Distribution of blood groups in CAD cases and controls.
*by chi-square test

among cases and Le (a-b+) was the most prevalent phenotype (66.9%) followed by Le (a-b-) (32.6%) in controls. There was a

Lewis Phenotype	Cases		Controls		p-value
	Number	Percentage	Number	Percentage	
Le (a-b-)	115	61.5%	61	32.6%	0.001*
Le (a-b+)	69	37.0%	125	66.9%	
Le (a+b-)	2	1.0%	1	0.5%	
Le (a+b+)	1	0.5%	0	0	
Total	187	100%	187	100%	

[Table/Fig-2]: Distribution of Lewis phenotype with CAD cases and controls.
*Significant by chi-square test

significant association of Le (a-b-) phenotype with CAD patients.

The [Table/Fig-3] shows frequencies of various risk factors in CAD patients with ABO and Lewis blood groups. Smoking (81.2%) followed by hypertension (39%) was the most common risk factor seen in CAD cases. The [Table/Fig-4] shows significant association of hypertension as a risk factor for CAD patients with blood group A (p-value=0.02) and no significant association with Lewis blood groups. The [Table/Fig-5] shows that there was no significant association of diabetes mellitus as a risk factor for CAD patients with ABO blood groups but shows significance with Lewis phenotype Le (a-b-) and Le (a-b+). The [Table/Fig-6] shows that there was a significant association of dyslipidemia as a risk factor for CAD patients with blood groups A and B and also with Lewis phenotype Le (a-b-) and Le (a-b+). The [Table/Fig-7] shows that there was a significant association of smoking as a risk factor for CAD patients with blood group A, however, except for Lewis phenotype Le (a-b+), it had significance with other three phenotypes. Multivariable logistic regression analysis showed that

Blood groups	Hypertension n (%)	Diabetes n (%)	Dyslipidemia n (%)	Smoking n (%)	Family History of CAD n (%)
O	28 (38.4)	22 (40)	20 (40)	66 (43.4)	14 (28)
B	22 (30.1)	21 (38.2)	8 (16)	53 (34.9)	5 (10)
A	22 (30.1)	11 (20)	22 (44)	26 (17.1)	31 (62)
AB	1 (1.4)	1 (1.8)	0 (0)	7 (4.6)	0 (0)
Total (% among 187)	73 (39.03%)	55 (29.41%)	50 (26.73%)	152 (81.28%)	50 (26.73%)
Le (a-b-)	40 (54.8)	45 (81.8)	38 (76)	100 (65.8)	34 (68)
Le (a-b+)	32 (43.8)	10 (18.2)	12 (24)	52 (34.2)	16 (32)
Le (a+b-)	1 (1.4)	0 (0)	0 (0)	0 (0)	0 (0)
Le (a+b+)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Total (% among 187)	73 (39.03%)	55 (29.41%)	50 (26.73%)	152 (81.28%)	50 (26.73%)

[Table/Fig-3]: Frequency of various risk factors with ABO and Lewis blood groups in cases.

Blood groups	CAD cases with hypertension n (%)	CAD cases without hypertension n (%)	p-value
O	28 (38.4)	49 (43)	0.53
B	22 (30.1)	40 (35)	0.48
A	22 (30.1)	19 (17)	0.02*
AB	1 (1.4)	6 (5)	0.33
Le (a-b-)	40 (54.8)	75 (65.8)	0.13
Le (a-b+)	32 (43.8)	37 (32.5)	0.11
Le (a+b-)	1 (1.4)	1 (0.8)	0.74
Le (a+b+)	0 (0)	1 (0.8)	0.42

[Table/Fig-4]: Association of hypertension between ABO and lewis blood groups.
*Significant by chi-square test

Blood groups	CAD cases with diabetes mellitus n (%)	CAD cases without diabetes mellitus n (%)	Total n (%)	p-value
O	22 (40)	55 (41.7)	77 (41)	0.83
B	21 (38.2)	41 (31)	62 (33)	0.34
A	11 (20)	30 (22.7)	41 (22)	0.68
AB	1 (1.8)	6 (4.5)	7 (4)	0.37
Le (a-b-)	45 (81.8)	70 (53)	115 (61.4)	<0.001*
Le (a-b+)	10 (18.2)	59 (44.7)	69 (37)	0.001*
Le (a+b-)	0 (0)	2 (1.5)	2 (1.1)	0.35
Le (a+b+)	0 (0)	1 (0.8)	1 (0.5)	0.51

[Table/Fig-5]: Association of diabetes mellitus between ABO and lewis blood groups.
*Significant by chi-square test

Blood groups	CAD cases with dyslipidemia n (%)	CAD cases without dyslipidemia n (%)	Total n (%)	p-value
O	20 (40)	57 (42)	77 (41)	0.84
B	08 (16)	54 (39)	62 (33)	0.003*
A	22 (44)	19 (13.9)	41 (22)	<0.001*
AB	0 (0)	7 (5.1)	7 (4)	0.10
Le (a-b-)	38 (76)	77 (56.2)	115 (61.4)	0.014*
Le (a-b+)	12 (24)	57 (41.5)	69 (37)	0.027*
Le (a+b-)	0 (0)	2 (1.5)	2 (1.1)	0.39
Le (a+b+)	0 (0)	1 (0.8)	1 (0.5)	0.54

[Table/Fig-6]: Association of dyslipidemia between ABO and lewis blood groups.
*Significant by chi-square test

Blood groups	CAD cases with smoking n (%)	CAD cases without smoking n (%)	Total n (%)	p-value
O	66 (43.4)	11 (31.4)	77 (41)	0.19
B	53 (34.9)	09 (25.7)	62 (33)	0.30
A	26 (17.1)	15 (42.9)	41 (22)	0.001*
AB	7 (4.6)	0 (0)	7 (4)	0.19
Le (a-b-)	100 (65.8)	15 (42.8)	115 (61.4)	0.01*
Le (a-b+)	52 (34.2)	17 (48.6)	69 (37)	0.11
Le (a+b-)	0 (0)	2 (5.7)	2 (1.1)	0.003*
Le (a+b+)	0 (0)	1 (2.9)	1 (0.5)	0.03*

[Table/Fig-7]: Association of smoking between ABO and lewis blood groups.
*Significant by chi-square test

Variables	Odds Ratio (95% CI)	p-value
ABO blood group	0.16 (0.09-0.20)	0.713
Lewis phenotype	1.71 (1.50-1.89)	<0.001*
Hypertension	1.51 (1.26-1.87)	<0.001*
Diabetes	1.34 (1.11-1.72)	<0.001*
Dyslipidemia	1.50 (1.23-1.96)	<0.001*
Smoking	1.48 (1.02-1.76)	<0.001*

[Table/Fig-8]: Multivariable logistic regression analysis for predicting risk factors for CAD.
*Significant by Multivariable Logistic Regression Analysis

Lewis phenotype was independently associated with an increased risk of CAD (Odds ratio: 1.71; 95% CI: 1.50-1.89) as shown in [Table/Fig-8].

DISCUSSION

The present objective was to study the pattern and association of patient's ABO and Lewis blood group phenotypes in clinically confirmed CAD patients. In present study, Blood group O was the most common (41.2%) blood group in the controls followed by 33.2% group B, 21.9% group A, 3.7% group AB. The prevalence of blood group O in the CAD patients was almost similar to that seen

in the controls, but comparatively, the frequency of non O groups' i.e., A, B groups showed a mild increase in the CAD patients; whereas the frequency of blood group AB in these patients was comparatively less than the controls.

In study, conducted at Punjab Institute of Cardiology, Lahore, by Sharif S et al., showed that the subjects with blood group A had significantly higher risk of developing CAD as compared to other blood groups [15]. Study done by Whincup PH et al., found that the incidence of CAD was higher in blood group A individuals than with non-A [16]. Study by Garg P et al., reported that there was a significant association between CAD and blood group B, where as Banerjee S et al., indicated incidence of CAD highest in blood group A [6,8].

The association of CAD with ABO blood group was supported by evidence indicating that elevated Von-willebrand factor (vWF)-Factor VIII levels are risk factors for CAD [15]. Blood group O individuals have approximately 25% lower plasma level of both factor VIII and vWF than other groups conferring an increased risk of thrombosis and CAD in non-O blood groups [17].

Wu O et al., performed a systematic review and meta-analysis of studies reporting the association of non-O blood groups with a variety of vascular disorders and observed a consistent relation between non-O blood group and an increased CAD risk [18]. He M et al., conducted a meta-analysis of data from the health professionals and they concluded that individuals with non-O blood group had an 11% increased risk of developing CAD compared to that in O blood group individuals [19].

In contrast to previous studies, Lutfullah AB et al., showed that there was no direct correlation between ABO blood groups and major CAD risk factors [20]. Amirzadegan A et al., investigated a possible relationship of ABO blood groups with a large number of CAD patients. They observed that there was no relationship between ABO blood groups and development of CAD. Moreover, the incidence of major risk factors was found equal in patients with different blood groups [21]. Present study also does not show a statistically significant association with ABO blood groups. These variations could be a result of biological variations or could be because of small sample size. A much larger study population may fully elucidate these findings.

In the present study, we observed that 94.1% of cases were associated with risk factors like smoking, hypertension, diabetes mellitus, dyslipidemia. Sharif S et al., showed the overall prevalence of hypertension was comparatively more with 58.5% and diabetes in 53.5% [15]. In the present study group of 187 patients, 73 (39.0%) had hypertension with a significant association with blood group A and 55 (29.4%) had associated Type II diabetes mellitus, however it did not show any significant association with ABO Blood groups.

Sayed EL and Amin HK, showed that hypertension was more common in blood group B followed by Blood group A [22]. They observed that blood group O is protective against hypertension and blood group A and B is protective against the diabetes, and hyperlipidemia. Chandra T and Gupta A, showed that blood group B persons were more susceptible to hypertension [23]. In contrast to above studies, in the present study, hypertension was seen more in blood group O (38.4%).

Blood Group A has been reported to show high levels of Serum Total cholesterol (TC) and Low Density Lipoproteins (LDL). Several studies showed high TC, Triglycerides (TG), LDL, and lower High Density Lipoproteins (HDL) in blood groups A and B while groups O and finally AB showed low levels of TC, TG, and LDL [24]. The present study showed a significant association with A and B blood groups. Smoking was seen in 54% in a study done by Sharif S et al., where as in present study, it was seen in 81.2% [15].

Epidemiological studies have suggested that the Lewis (a-b-) phenotype was an independent predictor of coronary heart disease [25]. Mechanisms underlying this association were not established. The Lewis negative phenotype is fairly common. Its prevalence in Caucasians was approximately 10% and considerably higher in black individuals [26,27]. In the present study, the prevalence of Le (a-b-) phenotype has been observed to be 61.5% whereas a report from Dhaka has shown its prevalence as 49.2% [28]. Chaudhury R and Shukla JS, observed that there was an increased frequency of Le (a-b-) (29.1%) phenotype among Indian patients with CAD [10]. Present study showed a significant association between this Lewis Phenotype and CAD (p-value=0.001).

In the Copenhagen male study and NHLBI family heart study, the Le (a-b-) phenotype was found to put the patients at an increased risk for CAD but the underlying mechanisms were unclear [29]. Kidambi TD, carried out a research on a hypothesis that Le (a-b-) phenotype was associated with high RBC aggregation and had a higher plasma viscosity which is known to be risk factors for CAD. He observed a significance difference in Plasma viscosity and RBC aggregation between patients with Lewis negative and Positive phenotype [30].

Elmgren A et al., found a higher triglyceride concentration in the Lewis (a-b-) men and it was suggested to be an independent risk for CAD [25]. In the present study, Le (a-b-) negative phenotype with Hypertension was found in 54.8% and showed no significant association with CAD patients. Petit JM et al., observed a non-significant increased triglycerides concentration [31]. In present study, among CAD cases with dyslipidemia, 76% had Lewis negative phenotype. The Le (a-b-) was observed more frequently in diabetics than the controls (29% Vs 10%) [26]. In the present study Lewis Negative phenotype with diabetes was observed in 81.8% and showed a significant association with CAD patients.

The Lewis Antigens play a well defined role in selectin-mediated cell adhesion events, and in inflammation and defense against infection. In the absence of these antigens, as in Le (a-b-), inflammation and chronic infection in turn contribute to the multifactorial pathogenesis of coronary disease events [32]. This might be a reason in present patients who were Le (a-b-) in getting CAD.

LIMITATION

Sample size was restricted due to the increased cost of the Lewis antisera. The relationship of the ABO and Lewis blood group with CAD independent of the major conventional risk factors could not be carried out as the number of patients with CAD without associated risk factors was too small. Further studies should be done involving larger number of patients to find high level of evidence regarding association of ABO blood group with CAD.

CONCLUSION

The results of present study showed a mild increased predisposition of blood group A and B individuals to CAD when compared to controls. However, the present study failed to show a significant association of ABO blood group with CAD. However, the study showed a significant association of ABO blood group with risk factors like hypertension, dyslipidemia and smoking. The present study also showed a significant association of Le (a-b-) phenotype with CAD and also with risk factors like diabetes mellitus, dyslipidemia and smoking. Screening for Lewis phenotype might help in taking primordial prevention steps against CAD.

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