

Association of coronary artery disease with Lewis and ABO blood group phenotypes- A case control study

Investigator

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Guide

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Professor and Head

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Co-guide

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Associate Professor

Department of Cardiology

Jubilee Mission Medical College & Research Institute, Thrissur

COMMUNICATION ADDRESS

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From,

Dr. Sree Raj.V
Junior Resident
Department of Immunohematology & Transfusion Medicine
Jubilee Mission Medical College & Research Institute
Thrissur

To,

The Chair Person
Institutional Ethics Committee
Jubilee Mission Medical College & Research Institute
Thrissur

Sir,

Sub: Application for institutional ethics committee approval: reg

I am enclosing herewith the detailed protocol of my research work entitled "Association of coronary artery disease with Lewis and ABO blood group phenotypes- A case control study" for the favour of approval from Institutional Ethics Committee.

Yours faithfully



Dr. Sree Raj.V

Place :Thrissur
Date :14/08/2019



APPLICATION FOR ETHICS COMMITTEE APPROVAL

1. Title of the research project : Association of coronary artery disease with Lewis and ABO blood group phenotypes- A case control study
2. Name, Designation, Qualification & Experience of the principal investigator : Dr. Sree Raj.V,
M.B.B.S
Junior Resident
Department of Immunohematology &
Transfusion Medicine
Jubilee Mission Medical College & Research
Institute, Thrissur
3. Name, Designation, Qualification & Experience of the guide : Dr. Susheela Jacob Innah
MBBS, MD (Pathology), DCP
Professor and Head
Department of Immuno Hematology &
Transfusion Medicine.
Jubilee Mission Medical College & Research
Institute, Thrissur
20yrs
4. Name, Designation, Qualification & Experience of the co-guide : Dr. Bino Benjamin
MBBS, MD, DM (Cardiology)
Associate Professor
Department of Cardiology
Jubilee Mission Medical College & Research
Institute, Thrissur
12 yrs
5. Name & address of the funding agency : Jubilee Mission Medical College & Research
Institute, Thrissur
6. Duration of the scheme : One and a half year

Place : Thrissur
Date : 14/08/2019

ETHICS COMMITTEE CONCERNS

1. Are the risks to the subjects minimised by using procedures which are consistent with sound research design and which do not unnecessarily expose the subjects to the risk and whenever appropriate, by procedures already being performed on the subjects for diagnostic purpose?
 - N/A

2. Are the risk to the subjects reasonable in relation to
 - a) The benefits that might be reasonably be expected as an outcome to the subjects/others.
 - N/A

 - b) Provision for free treatment for research related injury.
 - N/A

 - c) Compensation of subjects for disability or death resulting from such injury
 - N/A

 - d) The importance of the knowledge that may reasonably be expected to result from the research project
 - N/A

3. Will informed consent be sought from each positive subject /legally authorised representative?
 - YES

4. Does the research plan make adequate provisions for confidentiality and monitoring the data collected to ensure the safety of subjects?
 - YES

DECLARATION OF THE PRINCIPAL INVESTIGATOR

I hereby agree to abide by the rules and regulations of the institutional ethics committee and those of Jubilee Mission Medical College & Research Institute, Thrissur. I will not modify any aspect of the research project without prior approval from the ethics committee.

Place : Thrissur

Date : 14/08/2019

Signature :



Dr. Sree Raj.V
Junior Resident
Department of Transfusion Medicine
J.M.M.C & R.I, Thrissur

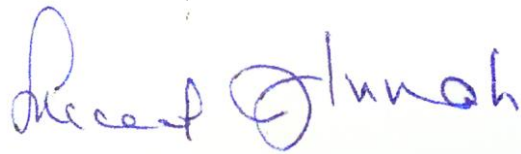
DECLARATION OF GUIDE

I hereby agree to guide the principal investigator throughout the period of research as per the protocol submitted for approval. I have necessary qualification and experience to guide this project.

Place : Thrissur

Date : 14/08/2019

Signature :



Dr. Susheela Jacob Innah
Professor & Head
Department of Transfusion Medicine
J.M.M.C & R.I, Thrissur

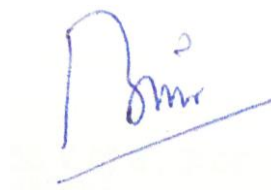
DECLARATION OF CO-GUIDE

I hereby agree to guide the principal investigator throughout the period of research as per the protocol submitted for approval. I have necessary qualification and experience to guide this project.

Place : Thrissur

Date : 14/08/2019

Signature :



Dr. Bino Benjamin
Associate Professor
Department of Cardiology
J.M.M.C & R.I, Thrissur

Introduction

Epidemiologic studies have found the Lewis negative phenotype to be independently associated with a two-fold higher prevalence of coronary artery disease and four-fold fatal coronary events^[1]. Lewis (a-b-) blood group has been reported to be associated with hypercholesterolemia, diabetes mellitus and insulin resistance conditions which eventually lead to CAD^[2]. Many studies have also shown the relationship between blood groups and different manifestations of cardiovascular disease^[3].

Different clinical studies have shown that blood A phenotypes are more susceptible to coronary artery disease^[3]. The major one being the Framingham study^[2].

In contrast, Garg P et al. reported a significant association between CAD and blood group B. while Sahita P et al. from Gujarat, India, did not observe any association between blood group and CAD. Cakir B et al reported a non- significant association between Lewis genotype and subclinical atherosclerosis and Saloma 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.

There is no literature from Kerala correlating Lewis antigen and ABO blood group with CAD, hence the present study to focus on the pattern and association of ABO and Lewis blood group phenotypes in clinically confirmed CAD patients.

Rationale

The present study aims to evaluate the association between Lewis antigen, ABO blood group, and coronary artery disease. This may allow identifying subjects at increased risk for coronary artery disease (CAD) and subsequently leading to more focused preventive measures and eventually to a specific treatment.

Review of literature

Coronary artery disease in Kerala is progressive and degenerative resulting in death at a very young age. The prevalence of definite CAD in Kerala increased nearly three times since 1993 without any difference in urban and rural areas. Most risk factors of CAD are highly prevalent in the state^[4] Hence early detection of these risk factors is highly essential to curtail CAD.

The ABO and Lewis blood group systems have both been linked to ischaemic heart disease (IHD)^[5].

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^[2]. Like the ABO blood group, the Lewis blood group reflects the actions of two distinct glycosyltransferases, FUT2/Secretor and Lewis/FUT3, an α 1,3/4-fucosyltransferase^[5].

T. Alexy et al. reported hypertriglyceridemia, insulin resistance, and higher prevalence of non-insulin dependent diabetes mellitus in the Le(a-b-) population ^[1]. M.N.Hasan et al revealed a higher prevalence of CAD in patients with blood group B ^[3].

In a study conducted by Enevold et al, no significant associations were observed between Lewis blood group/ABO blood groups and hypertension, IHD, or obesity ^[5]. The meta-analysis by Zhuo Chen et al suggests that blood group A and non-O are associated with an increased risk of CAD ^[6]. A study by Sharif S et al showed that subjects with blood group A had a significantly higher risk of developing IHD compared to other blood groups ^[7].

Materials and methods

Research question:

Does ABO and Lewis blood group phenotypes have an association with Coronary Artery Disease?

Aim and Objectives:

Primary objective

- To study the association of Lewis antigen and ABO blood group phenotypes with CAD patients attending the tertiary care hospital in Kerala.

Secondary objective

- To find the association of Lewis antigen and ABO blood group phenotypes with smoking, alcohol use, diabetes, hypertension and dyslipidemia.

Study design

Hospital-based case-control analytical study

Study setting and duration:

The Study will be conducted over a period of eighteen months in the Department of Transfusion Medicine in association with Department of Cardiology at Jubilee Mission Medical College and Research Institute, Thrissur, a tertiary care multispecialty teaching hospital in central Kerala.

Sampling:

- **Sample size**

Based on the proportion of Le(a-b-) observed in an earlier publication^[2] with 95% confidence level and 90% power, minimum sample size comes to 70 in cases and 140 in control group

$$n = \frac{[Z_{1-\alpha/2} \cdot \sqrt{2p(1-p)} + Z_{1-\beta} \sqrt{p_1(1-p_1) + p_2(1-p_2)}]^2}{(p_1 - p_2)^2}$$

- **Inclusion criteria:**

- Patients in the age group of 30-55 years at the time of data collection
- Diagnosed with coronary artery disease on the basis of positive changes in coronary angiogram (More than 70% coronary stenosis)

- **Exclusion Criteria:**

- Patients with other systemic illnesses like malignancy, autoimmune diseases.
- Patients who are not willing to participate in the study.

- **Sampling procedure:**

First 70 cases attending the Cardiology department which satisfy the inclusion and exclusion criteria will be included in the study.

- **Method of data collection**

70 cases attending the Cardiology department which satisfy the inclusion and exclusion criteria will be taken as cases. 140 age and gender matched healthy blood donors who are eligible to donate blood as per the inclusion and exclusion criteria of Drugs and Cosmetics Rules, 1945 are taken as controls.

After taking written, informed consent from the patients and blood donors satisfying the inclusion criteria, 3 mL blood is collected from cubital vein in Ethylene Diamine Tetra Acetic acid (EDTA) tubes under aseptic precautions. Samples are collected separately from each CAD patient as well as from blood donors.

Collection of blood samples repeatedly from the same subjects are 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 the required quantity.

Lipid profile values, fasting and post prandial blood sugar values will be collected from the laboratory information system. Blood pressure values will be obtained from the medical record and donor forms.

Diabetes mellitus is defined as fasting serum glucose >126 mg/dL and or the reported use of diabetic medications^[8]. Hypertension is defined as systolic blood pressure (BP) >140 or diastolic BP >90 mmHg, or reported use of antihypertensive medications^[8].

Alcohol use pattern is categorised as none, low/moderate use (1–14 drinks/week), and heavy use (>14 drinks/week)^[9].

Smoking pattern is categorised as Smoker (An adult who has smoked at least 100 cigarettes in his or her lifetime, and who now smokes every day), Ex smoker (An adult who has smoked at least 100 cigarettes in his or her lifetime but who had quit smoking), Never smoker: An adult who has never smoked, or who has smoked less than 100 cigarettes in his or her lifetime^[9].

ABO blood grouping is performed according to department's Standard Operational Procedures (SOP). Commercially available monoclonal blood group antisera are used for forward grouping; for reverse grouping 5% pooled suspension of A, B and O cells are used which are prepared according to SOP.

Lewis antigen typing is done by using monoclonal antisera as per manufacturer's instructions.

Statistical analysis

Statistical analysis will be carried out using SPSS version 25.0. Descriptive Statistics for the categorical variables are 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 is done using chi-square test.

Estimated expenditure

Establishment

- Antisera : ₹ 50000/-
- Vacutainers : ₹ 1700/-

Equipment : nil

Research literature : ₹ 1000

Cost of preparation of research papers : ₹ 1000

Expenditure will be met by the institution.

Ethical Considerations

No ethical issues expected. Study will be conducted only after the approval of the ethical committee.

References

1. Alexy T, Pais E, Wenby RB, Mack WJ, Hodis HN, Kono N, Wang J, Baskurt OK, Fisher TC, Meiselman HJ. Abnormal blood rheology and chronic low grade inflammation: possible risk factors for accelerated atherosclerosis and coronary artery disease in Lewis negative subjects. *Atherosclerosis*. 2015 Mar 1;239(1):248-51.
2. Madithadu A, Rajendran A, Durgaprasad R, Jothibai S. Association of Coronary Artery Diseases with ABO and Lewis Blood Group Phenotypes at a Tertiary Care Teaching Hospital in Southern India. *Journal of Clinical & Diagnostic Research*. 2018 Jul 1;12(7).
3. Hasan MN, Chowdhury AW, Islam LM, Safiuddin M, Haque M, Hoque H. Association of ABO blood group with CAD in patients undergoing CAG in Cardiology Department of Dhaka Medical College and Hospital. *University Heart Journal*. 2014;10(2):81-4.
4. Krishnan MN, Zachariah G, Venugopal K, Mohanan PP, Harikrishnan S, Sanjay G, Jeyaseelan L, Thankappan KR. Prevalence of coronary artery disease and its risk factors in Kerala, South India: a community-based cross-sectional study. *BMC cardiovascular disorders*. 2016 Dec;16(1):12.
5. Enevold C, Nielsen CH, Molbo D, Lund R, Bendtzen K, Fiehn NE, Holmstrup P. Lewis and ABO blood group-phenotypes in periodontitis, cardiovascular disease, obesity and stroke. *Scientific reports*. 2019 Apr 18;9(1):6283.
6. Chen Z, Yang SH, Xu H, Li JJ. ABO blood group system and the coronary artery disease: an updated systematic review and meta-analysis. *Scientific reports*. 2016 Mar 18;6:23250.
7. Sharif S, Anwar N, Farasat T, Naz S. ABO blood group frequency in Ischemic heart disease patients in Pakistani population. *Pakistan journal of medical sciences*. 2014 May;30(3):593.
8. Theodore A. Kotchen. Hypertensive vascular diseases. In:James f Shanahan, Kim J david, editors. *Harrison's principles of internal medicine*. New York: McGraw-Hill; 2011. p. 2047-59.
9. Kelly SG, Plankey M, Post WS, Li X, Stall R, Jacobson LP, Witt MD, Kingsley L, Cox C, Budoff M, Palella Jr FJ. Associations between tobacco, alcohol, and drug use with coronary artery plaque among HIV-infected and uninfected men in the multicenter AIDS cohort study. *PloS one*. 2016 Jan 26;11(1):e0147822.

SI No: _____

Date : _____

PROFORMA

CASE	
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CONTROL	
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Name :

Age:

Sex:

Hosp Number:

Address :



Presenting complaints :

Past history :

Personal history :

Tobacco use ☐ _____

Alcohol ☐ _____

Physical Examination

Height: _____ Kg

Weight: _____ cm

BMI: _____

Investigations

Angiogram : _____

Hypertension: <input type="checkbox"/> yes <input type="checkbox"/> no		On medication: <input type="checkbox"/> yes <input type="checkbox"/> no	
BP: _____ mmHg			
Lipid profile: Total Cholesterol : _____ mg/dL TG : _____ mg/dL HDL : _____ mg/dL LDL : _____ mg/dL		Diabetes: <input type="checkbox"/> yes <input type="checkbox"/> no On medication <input type="checkbox"/> yes <input type="checkbox"/> no PPBS : _____ mg/dL FBS : _____ mg/dL	

Blood Group			
A	B	AB	O

Rh	
+	-

Le ^a	
+	-

Le ^b	
+	-

PATIENT INFORMATION SHEET

1. Name of the study:
 - Association of coronary artery disease with Lewis and ABO blood group phenotypes- A case control study.
2. Expected duration of participation in the study:
 - Duration of hospital stay
3. Expected benefits of the study:
 - Better detection and prevention of coronary artery disease
4. Risks associated with the study:
 - Nil
5. Confidentiality of documents:
 - All information regarding the patient will be kept confidential
6. Treatment of research related injuries:
 - Not applicable
7. Compensation of study related disability or death:
 - Not applicable
8. Freedom to withdraw from the study without penalty:
 - Yes
9. Expenses for collecting and testing samples:
 - No additional expense
10. Research related expense:
 - No additional expense
11. Place of study:
 - Jubilee Mission Medical College & Research Institute, Thrissur
12. Name and address of the investigator:
 - Dr. Sree Raj.V
Junior Resident
Department of Transfusion Medicine
Jubilee Mission Medical College & Research Institute, Thrissur
☎ 9495018785

PATIENT CONSENT FORM

Research title:

'Association of coronary artery disease with Lewis and ABO blood group phenotypes- A case control study'

I have read and understood the patient information sheet. I understand that I have the opportunity to ask questions. The investigator has explained the type, objective, scope, benefits, duration and other relevant details regarding the study.

The investigator has clearly explained the rare complications that can arise during blood collection. I am willing to undergo medical examination and to provide necessary data and the required blood samples for the estimation of blood sugar, lipid profile, blood group and Lewis phenotyping.

I know that I will have to bear no extra cost for participating in the study. I am agreeing to participate in this study at my own will and I understand that I don't have any obligation to participate in this study.

I also know that I can withdraw from this study at any time without providing any explanation and that my withdrawal from the study will not affect the treatment or rights given to me.

I have been assured that my medical records will be kept confidential and no personal reference will be made in the study data. I hereby give permission to any authorized persons to analyse the data collected from me.

After reading and understanding all the conditions given above I give my consent to participate in this study.

Date :

Place : Thrissur

Name :

Address :

Name of the investigator:

Dr. Sree Raj. V

Junior resident

Department of Transfusion Medicine

Jubilee Mission medical College

Thrissur

☎ 9495018785

Sign:

Sign:

CONSENT FORM (controls)

Research title:

'Association of coronary artery disease with Lewis and ABO blood group phenotypes- A case control study'

The investigator has explained that the objective of this study is to investigate the association of coronary artery disease with Lewis and ABO blood group phenotypes. I understand that this study has two groups, Patients with coronary artery disease and people without the disease and that I am invited to be part of the second category.

I am willing to undergo medical examination and to provide necessary data and the required blood samples for the blood grouping and Lewis phenotyping. The investigator has clearly explained the rare complications that can arise during blood collection.

I know that I will have to bear no extra cost for participating in the study. I have been assured that my medical records will be kept confidential and no personal reference will be made in the study data. I hereby give permission to any authorized persons to analyse the data collected from me.

I am agreeing to participate in this study at my own will and I understand that I don't have any obligation to participate in this study. I also know that I can withdraw from this study at any and that my withdrawal from the study will not affect me in any ways.

After reading and understanding all the conditions given above I give my consent to participate in this study.

Date :

Place : Thrissur

Name :

Address :

Name of the investigator:

Dr. Sree Raj. V

Junior resident

Department of Transfusion Medicine

Jubilee Mission medical College

Thrissur

☎ 9495018785

Sign:

Sign: