ORIGINAL ARTICLE

Association of Non-HDL Cholesterol, Homocysteine and Vitamin D in Acute Coronary Syndrome

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Abstract

Background: The role of Low Density Lipoprotein Cholesterol (LDL–C) in the pathogenesis of Coronary Heart Disease (CHD) has been well established. In many studies it was found that Non-HDL cholesterol (total cholesterol minus High Density Lipoprotein Cholesterol [HDL-C] is a better predictor of CHD risk than LDL-C alone. High homocysteine levels are associated with increased risk of cardiovascular and cerebrovascular disease. An inverse relation has been seen between vitamin D serum level and coronary artery calcification. Studies are inadequate among Indians to establish the role of these non-conventional risk factors in acute coronary syndrome.

Objectives: To correlate the values of non-HDL cholesterol, vitamin D and homocysteine in patients with acute coronary syndrome (ACS) with controls

Methods: For this retrospective study cases were the patients admitted in Poona Hospital and Research Centre between November 2013 and November 2015 with acute coronary syndrome whereas controls were patients admitted in Poona Hospital and Research Centre during the same period with diagnosis other than Acute coronary syndrome. Each patient was subjected to detailed clinical history, clinical examination and investigations such as lipid profile, serum homocysteine, and Vitamin D3. Unpaired t-test was used to compare the quantitative data whereas Chi-square test or Fisher's exact test was used for qualitative data.

Results: ACS group had significantly higher mean total serum cholesterol, mean LDL cholesterol, mean non-HDL cholesterol, and mean plasma homocysteine as compared to control group. However, there was no statistically significant difference between the two groups in Vit D levels. Odds ratio was maximum for Non HDL cholesterol, followed by LDL cholesterol, HDL cholesterol, Serum Homocysteine, and Total cholesterol

Conclusions: Non-HDL cholesterol was a better predictor of cardiovascular diseases than LDL-C, HDL-C or total cholesterol.

Introduction

Atherosclerotic vascular disease, which encompasses coronary heart disease (CHD), cerebrovascular disease, and peripheral arterial disease, is responsible for the majority of cases of cardiovascular disease (CVD) in both developing and developed countries. The Framingham Heart Study and many other large prospective cohort studies[1] have demonstrated the importance of major CVD risk factors in the appearance of vascular events. It is apparent, however, that a substantial proportion of cardiovascular events occur in individuals who exhibit none of these classic risk factors. This has led to an increasing interest in identifying novel biomarkers that might improve the global risk prediction of CVD. In recent years, a number of emerging markers have been proposed as significant predictors of atherosclerosis and its thrombotic complications.

The role of Low Density Lipoprotein Cholesterol (LDL–C) in the pathogenesis of CHD has been well established. This is the most well studied coronary risk factor.[2] In many studies it was found that Non-HDL cholesterol (total cholesterol minus High Density Lipoprotein Cholesterol [HDL-C]) is a better predictor of CHD risk than LDL-C alone.[3] Vitamin D deficiency is prevalent in most parts of the world. 25-hydroxyvitamin D is a marker of vitamin D status in the human body. Some population based studies have shown that 60%-65% of Indians have 25-hydroxyvitamin D deficiency.[4] Most body cells, including cardiomyocytes, vascular smooth muscles and the endothelium of the vessels have vitamin D receptors. Recent studies are indicative of a relation between vitamin D deficiency and cardiovascular disease, increased blood pressure, increased insulin resistance, heart failure and fatal strokes.[5]

High homocysteine levels are associated with increased risk of cardiovascular and cerebrovascular disease although there are studies that show no increase in risk and there is still debate as to the strength and validity of the association.

Studies are inadequate among Indians to establish the role of these non-conventional risk factors in acute coronary syndrome. Hence, an attempt is made in the present study to correlate the values of non-HDL cholesterol, vitamin D and homocysteine in patients with acute coronary syndrome with controls

Material and Methods

Cases were the patients admitted in Poona Hospital and Research Centre between November 2013 and November 2015 with acute coronary syndrome whereas controls were patients admitted

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Received: 19.07.2016; Accepted: 11.04.2018

Table 1: Characteristics of cases and controls

	ACS (N= 145)	Control (N = 145)	p value
Mean age (SD)	58.25 (± 12.28)	57.27 (± 11.42)	0.77
Gender (%)			
Males	85 (58.62)	88 (60.69)	0.811
Females	60 (41.38)	57 (39.31)	
BMI (%)			
< 23	44 (30.34)	65 (44.83)	0.015
≥ 23	101 (69.66)	80 (55.17)	
Sedentary lifestyle (%)			
Yes	114 (78.62)	103 (71.03)	0.176
No	31 (21.38)	42 (28.97)	
Diabetes mellitus (%)			
Yes	52 (35.86)	51 (35.17)	0.999
No	93 (64.14)	94 (64.83)	
Hypertension (%)			
Yes	67 (46.21)	57 (48.28)	0.814
No	78 (53.79)	75 (51.72)	
Smoking (%)			
Yes	23 (15.86)	10 (6.9)	0.025
No	122 (84.14)	135 (93.1)	

Table 3: Odds ratio cases and controls

Laboratory investigations	ACS (N= 145)	Control (N = 145)	Odds ratio (95 % CI)
TC (%)			
< 200 mg/dl	104 (71.72)	132 (91.03)	4.00
≥ 200 mg/dl	41 (28.28)	13 (8.97)	(2.03 – 7.86)
LDL (%)			
< 100 mg/dl	15 (10.34)	88 (60.69)	13.38
≥ 100 mg/dl	130 (89.66)	57 (39.31)	(7.13 – 25.12)
Non-HDL (%)			
< 130 mg/dl	11 (7.59)	88 (60.69)	18.80
≥ 130 mg/dl	134 (92.41)	57 (39.31)	(9.34 – 37.84)
HDL (%)			
< 40 mg/dl	117 (80.69)	54 (37.24)	7.04
≥ 40 mg/dl	28 (19.31)	91 (62.76)	(4.13-11.99)
Vit D level (%)			
< 30 ng/ml	119 (82.07)	116 (80.0)	1.14
≥30 ng/ml	26 (17.93)	29 (20.0)	(0.64 – 2.06)
Plasma Homocysteine (%)			
< 15 µmol/L	38 (26.21)	102 (70.34)	6.67
≥15 µmol/L	107 (73.79)	43 (29.66)	3.99 – 11.16

Table 2 : Laboratory investigations in cases and controls

Laboratory investigations	ACS (N= 145)	Control (N = 145)	p value
Mean total serum cholesterol (SD)	193.90 (±23.98)	155.86 (±14.90)	< 0.01
Mean LDL cholesterol (SD)	120.41 (±16.72)	89.83 (±12.23)	< 0.01
Mean non-HDL cholesterol (SD)	159.02 (±24.20)	114.74 (±13.57)	< 0.01
Vit D level (%)			
< 30 ng/ml	119 (82.07)	116 (80.0)	0.437
≥30 ng/ml	26 (17.93)	29 (20.0)	
Mean plasma homocysteine µmol/L (SD)	14.69 (±6.68)	12.00 (±3.63)	< 0.01

in Poona Hospital and Research Centre during the same period with diagnosis other than Acute coronary syndrome. For this retrospective study, cases and controls were included who were ready to participate. Permission was obtained from Institutional Ethics Committee (IEC), Scientific advisory committee of the institution and written informed consent was taken from all the participants.

Based on previously published studies[6] setting an alpha error at 0.05, and power at 80%, sample size of 145 was calculated for each group by formula.[7]

Inclusion criteria

1. Acute Coronary Syndrome (ACS) patients with

   Unstable angina: Severe chest discomfort with at least one of the three features

   A. It occurs at rest (or with minimal exertion), lasting >10 minutes.

   B. It is of relatively recent onset (i.e., within the prior 2 weeks).

   C. It occurs with a crescendo pattern (i.e., distinctly more severe, prolonged, or frequent than previous episodes).

   Non-ST elevation MI: A patient with unstable angina with evidence of myocardial necrosis, as reflected in abnormally elevated levels of biomarkers of cardiac necrosis.

   ST elevation MI- Angina with objective ECG evidence of ST elevations of at least 1 mm in limb leads and 2 mm in precordial leads with raised cardiac enzymes.

Exclusion Criteria

Valvular heart disease, peripartum cases, patients on lipid lowering drugs and patients with previous history of coronary heart disease.

Selection of Controls

A control group comprising of individuals visiting Blood Bank for blood donation, patients visiting OPD or patients admitted in Poona Hospital and Research Centre with diagnosis other than acute coronary syndrome. The controls were matched with cases for age,gender and co morbid conditions such as Diabetes mellitus, Hypertension

Data Collection

Each patient was subjected to detailed clinical history, clinical examination and Investigations as per the study proforma. Blood for serum homocysteine and lipid profile was taken in fasting state. Blood for Vitamin D3 was taken irrespective of the time of the meal. The samples were collected within 24 hours of onset of ACS.

Patients with BMI ≥23 kg/m^2 were considered overweight / obese.[8]

Definition for hypertension by JNC VII (Joint National Committee) -defined as a systolic blood pressure >140 mmHg and/or a diastolic blood pressure >90 mmHg based on the average of 2 blood pressure measurements at the time of admission[9], or a patient's self-reported history of hypertension or antihypertensive drug use, supported by documents. Although definition of hypertension was not specifically addressed in JNC VIII guidelines, thresholds were adopted for the treatment of blood pressure that are generally consistent with the definitions provided by JNC VII.[10]

Diabetes was diagnosed if fasting plasma glucose was >126 mg/dL or 2 hours post prandial > 200 or HbA1c>6.5 or patient was on anti-diabetic medications. (According to ADA guidelines)[11]

Smokers were defined as those smoked daily. Ex-smokers and occasional smokers were classified as nonsmokers.

Expected values of total cholesterol (NCEP ATP III)

Desirable: <200mg/dl

Borderline high: 200 – 239 mg/dl

High: ≥ 240 mg/dl

Expected values of LDL Cholesterol (NCEP ATP III) (in mg/dl)

<100: Optimal

100-129: Near optimal/above optimal

130-159: Borderline high

160-189: High

>190: Very high

Vit D Range [12]

Deficient: < 20 ng/ml

Insufficient: 20 – 29 ng/ml

Sufficient: 30 – 100 ng/ml

Potential toxicity: > 100 ng/ml

Homocysteine Range: 1.0-50.0 µmol/L

Normal values: < 15 µmol/L [13]

The statistical analysis was done by using SPSS (Statistical Package for Social Sciences) version 20.0 statistical software. Qualitative data variables were expressed by using frequency and Percentage (%). Quantitative data variables were expressed by using Mean and Standard deviation. Unpaired t-test was used to compare the quantitative data whereas Chi-square test or Fisher's exact test (Expected cell count is < 5) was used for qualitative data. P value of less than 0.05 was considered as significant.

Results

Between November 2013 and November 2015, 145 patients of ACS and equal number of controls were included in the study.

As depicted in Table 1, mean age, gender, sedentary lifestyle, presence of diabetes mellitus and high blood pressure were matched between study and control group whereas BMI and h/o smoking was not matched between the groups. Hence, BMI and smoking may be confounding variables for the present research.

It can be seen from Table 2, there was statistically significant difference between the two groups with respect to mean total serum cholesterol, mean LDL cholesterol, mean non-HDL cholesterol, and mean plasma homocysteine. ACS group had significantly higher mean total serum cholesterol, mean LDL cholesterol, mean non-HDL cholesterol, and mean plasma homocysteine as compared to control group. However, there was no statistically significant difference between the two groups in Vit D levels.

It can be seen from table 3 that odds ratio was maximum (18.8) for Non HDL cholesterol, followed by LDL cholesterol (13.38), HDL cholesterol (7.04), Serum Homocysteine (6.67), and Total cholesterol (4.00).

Discussion

Cardiovascular diseases are one of the major causes of morbidity and mortality in the contemporary society. Advances in our understanding of the ways in which the traditional cardiovascular risk factors interact to initiate atherosclerosis and promote the development of cardiovascular disease have enhanced our ability to assess risk in the individual patient. In addition, there is substantial interest in identifying new risk factors for cardiovascular disease, to improve our understanding of disease biology and to account for the cases of cardiovascular disease that cannot be explained by known risk factors. In recent years, debate has arisen regarding the validity and usefulness of these new measures.

In the present research 145 patients of ACS admitted in the study period with mean age of 58.25±12.28 years and 145 matched controls having mean age of 57.27 ±11.42 years were included. The ACS group had 58.62% male and 41.38% female patients whereas the control group had 60.69 % males and 39.31 % females. 78.62 % of ACS patients, and 71.03% of controls had sedentary lifestyle. Diabetes mellitus was present in 35.86% of patients with ACS and in 35.17 % of controls. 46.21 % of ACS patients had hypertension whereas 48.28% of controls had hypertension. In all above variables p value was more than 0.05, hence both the groups were matched in respect of age, gender, sedentary lifestyle, presence of diabetes mellitus and hypertension.

In our study, 69.66% patients with ACS had BMI ≥ 23kg/m^2 whereas 55.17% of controls had BMI ≥ 23kg/m^2. According to Kasama K et al., Asians with BMI ≥ 23kg/m^2 are considered to be at high risk.[8] 15.86 % of ACS patients were smokers whereas 6.9 % of controls were smokers. In these variables p value was less than 0.05, hence the two groups were not matched by BMI and smoking habit. These two variables were confounding variables in the present research.

Total cholesterol

Mean total serum cholesterol for ACS patients was 193.90 ± 23.98 whereas for controls it was 155.86 ± 14.90. Mean serum cholesterol was significantly elevated in patients of ACS as compared to controls. This is consistent with the study conducted by Nobilli et al. [14] where they found that total cholesterol was independently associated with risk of myocardial infarction. Odds ratio with Total cholesterol >200mg/dl as exposure was 4.00(95%CI, 2.03-7.86) for ACS patients.

Low Density Lipoprotein Cholesterol

Mean serum LDL C for patients of patients of ACS was 120.41 ± 16.72 mg/dl. It was significantly more as compared to LDL C of controls (89.83 ± 12.23 mg/dl). Similar results were reported by K Maruyama et al. in patients of ACS where mean serum LDL C levels were raised in patients of ACS as compared to controls.[15] Odds ratio with exposure as LDL cholesterol ≥100mg/dl was 13.38 (95 % CI, 7.13-25.12) in ACS patients .

Non-HDL Cholesterol

Mean Non-HDL Cholesterol was significantly higher in patients of ACS (159.02 ± 24.20 mg/dl) as compared to controls (114.74 ± 13.57mg/dl). A meta analysis from 68 studies [16] reported that non-HDL-C was the best predictor among all cholesterol measures, for Coronary artery disease events. Significantly raised non HDL cholesterol in patients presenting with ACS was reported in many other studies as well.[6,17,18] Odds ratio for non HDL cholesterol ≥130 mg/dl as exposure was 18.80(95% CI, 9.34 – 37.84) in ACS patients

Vitamin D

In the present study, it was found that insufficient vitamin D (< 30 ng/ml) was not an individual risk factor for acute coronary syndrome.82.07% of individuals in the ACS group had vitamin D < 30 ng/ml whereas 80% of individuals in control group had vitamin D < 30 ng/ml. Although Vitamin D was deficient in > 80 % of population, there was no statistically significant difference between the two groups.

Thomas J. Wang et al [19] studied 1739 Framingham Offspring Study participants (mean age 59 years; 55% women) without prior cardiovascular disease. They concluded that vitamin

Table 4: Descending order of odds ratio

	ACS
Non HDL-C	18.80 (95%CI, 9.34-37.84)
LDL-C	13.38 (95 % CI, 7.13-25.12)
HDL – C	7.04 (95 % CI, 4.13-11.99)
Homocysteine	6.67 (95 % CI, 3.99- 11.16)
Total cholesterol	4.00 (95 % CI, 2.03 – 7.86)
Vitamin D	1.14 (95 % CI, 0.64 – 2.06)

D deficiency was associated with incidence of cardiovascular disease. There is clear evidence that patients with cardiovascular disease have lower levels of 25(OH)D, but a similar association exists for a large number of other medical conditions like DM, hypertension, obesity etc., suggesting that this association may be confounded by reduced levels of physical activity and time spent outdoors in those with cardiovascular and other diseases. Odds ratio with Serum 25 (OH) D < 30ng/ml as exposure was 1.74 (95 % CI, 0.64 – 2.06) in ACS patients

At present, there is insufficient evidence to support vitamin D supplementation as a way of improving cardiovascular outcomes. However, many cardiovascular patients are frail and immobile and are at risk of markedly reduced vitamin D levels and osteoporosis. Supplementation of such patients is justified to prevent very low levels of 25(OH)D, with their squeal of musculoskeletal pain, myopathy and accelerated bone loss.

Homocysteine

In the present study we found that mean homocysteine was significantly higher in patients with ACS (14.69 ±6.68µmol/L) as compared to controls. (12.0 ±3.63µmol/L). Many studies have established a correlation between hyperhomocysteinemia and elevated risk for cardiovascular events, but the precise role of plasma homocysteine in cardiovascular disease is unclear. Plasma homocysteine increases with aging and is associated with smoking and diet patterns. Most of the studies conducted on effect of homocysteine on cardiovascular diseases reported an association of plasma homocysteine with the risk for cardiovascular events. Other studies reported that the effect disappears following adjustment for other risk factors, because homocysteine co segregates with other risk factors. It has been difficult to identify an independent effect of homocysteine on cardiovascular disease. Homocysteine can be modified to some extent by vitamin supplementation. Homocysteine reduction may have benefit in reducing cardiovascular risk particularly in the elderly. E Arnesen et al. concluded in their study that raised homocysteine was associated with myocardial infarction. (mean homocysteine of 12.7 µmol/L in cases vs 11.6 µmol/L in controls).[20] Odds ratio with serum homocysteine ≥ 15 µmol/L as exposure was 6.67 (95 % CI,3.99 – 11.16) in ACS patients.

In the present study, Non HDL cholesterol was a better predictor of ACS than others. Odds ratio in ACS patients was Non HDL- C (18.80), LDL-C (13.38), HDL C (7.04), Homocysteine (6.67) and Total cholesterol (4.0). Odds ratio was maximum for non HDL cholesterol followed by LDL C and total cholesterol. Hence, non HDL-C predicts cardiovascular risk better than LDL-C and total cholesterol in our study population. In our study non HDL C was the best predictor of cardiovascular risk. Non HDL C was followed by LDL C, low HDL C, homocysteine, and total cholesterol.

Limitations

Although we attempted to choose a control group that was matched with the cases with respect to most of the conventional cardiovascular risk factors, but smoking, and BMI could not be matched. Hence, they may act as confounding factors.

This study was conducted in a single tertiary care hospital and represents only a small population mostly urban.

Patients may have other cardiovascular risk factors like chronic inflammatory diseases, raised high sensitivity C- reactive protein, raised fibrinogen etc. which were not studied or ruled out.

Conclusions

Significant association was found between non-HDL – C, LDL cholesterol, Total cholesterol, and homocysteine with acute coronary syndrome

Non-HDL cholesterol was a better predictor of cardiovascular diseases than LDL-C, HDL-C or total cholesterol.

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