Role of ApoE gene polymorphism and nonconventional biochemical risk factors among very young individuals (aged less than 35 years) presenting with acute myocardial infarction

Mohit D. Gupta, M.P. Girish, Prattay G. Sarkar, Amit Gupta, Anand Kategari, Ankit Bansal, Rajni Saijpal, Vishal Batra, Manjari Rain, Sanjay Tyagi, Qadar Pasha

A R T I C L E   I N F O

Article history:
Received 1 May 2018
Accepted 12 August 2018
Available online 5 September 2018

Keywords:
Myocardial infarction
Cardiovascular disease
ApoE
Genetic polymorphism
Correlation

A B S T R A C T

Background: Incidence rate of acute myocardial infarction (MI) has increased in younger population over the years. The young patients have a different risk profile, presentation, and prognosis than the elderly. Hence, it is essential to understand the risk factors in young patients for proper treatment.

Methods: Apolipoprotein E (ApoE) polymorphism and biochemicals such as total cholesterol, serum triglycerides, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), apolipoprotein A1 (ApoA1), apolipoprotein B (ApoB), lipoprotein(a), insulin, interleukin-6, homocysteine, fibrinogen, and highly sensitive C-reactive protein were investigated in very young MI (yMI patients; age ≤ 35 years; n = 125), in old MI (oMI patients; age > 35 and < 80 years; n = 111), and healthy controls (age ≤ 35 years; n = 103).

Results: HDL-C was significantly lower in yMI patients than in controls (p = 2.63E-04) and oMI patients (p = 1.29E-05). ApoA1 was also lowest in yMI patients, but significant only in comparison to controls (p = 2.62E-04). The yMI group had the highest ratios of total cholesterol:HDL-C (p = 0.027 in yMI patients versus controls and p = 0.018 in yMI patients versus oMI patients), LDL-C:HDL-C (p = 0.002 in yMI patients versus controls and p = 0.005 in yMI patients versus oMI patients), and ApoB:ApoA1 (p = 8.75E-05 in yMI patients versus controls and p > 0.05 in yMI patients versus oMI patients). No significant pattern of ApoE polymorphisms was observed.

Conclusion: The lower level of HDL-C and ApoA1 and higher ratios of total cholesterol:HDL-C, LDL-C:HDL-C, and ApoB:ApoA1 are risk factors for MI in young patients.

© 2018 Cardiological Society of India. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Ischemic heart disease (IHD) is the leading cause of death globally, with India having a huge burden of associated morbidity and mortality. Among Indians, cardiovascular disease (CVD) manifests at least 5–6 years earlier than their western counterparts. Based on the treatment and outcomes of acute coronary syndromes in India (CREATE) registry, information collected between 2003 and 2005, the mean age of acute coronary syndrome (ACS) presentation was 57.5 years, which is 7–11 years younger than reports from Western literature. Dismally, young India is at the risk of CVD, especially in urban developments.

Myocardial infarction (MI) presenting at a young age shows a different clinical, angiographic, and pathophysiological profile compared with older age. These patients have a different risk profile, presentation, and prognosis. Angiographic data regarding the extent and severity of coronary artery disease (CAD) in the very young patients with manifest CAD is very limited. Moreover, abnormalities of metabolic, inflammatory, and thrombogenic biological systems would contribute to CAD presenting at a young age. Emergence of novel risk factors for CVD including hyperhomocysteinemia, lipoprotein(a) [Lp(a)], insulin level, highly sensitive C-reactive protein (hsCRP), interleukin-6 (IL-6), fibrinogen, and others among adults of different age groups may have the same clinical implications.

https://doi.org/10.1016/j.ihj.2018.08.013
0019-4832/© 2018 Cardiological Society of India. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
Understanding the clinical, pathophysiological, and angiographic differences between young and old patients is essential to treat the patients efficiently. We, therefore, studied the clinical, angiographic, and biochemical characteristics in very young MI (yMI) patients (age ≤ 35 years) and old MI patients (oMI) (age >35 and <80 years).

In addition, we also studied the influence of apolipoprotein E (ApoE) polymorphism, if any, on advancement and unfolding of MI with respect to age. ApoE is essential for catabolism and transport of lipoproteins. ApoE polymorphism is an eminent genetic determinant of cardiovascular risk. Three major isoforms or alleles of ApoE are known that differ from each other by only one or two amino acids at positions 112 and 158 and have different ApoE structures and functions. These are E2 (Cys112, Cys158), E3 (Cys112, Arg158), and E4 (Arg112, Arg158). The E3 allele is the major allele, while E2 and E4 are the variants. Both E2 and E4 alleles increase the risk for heart diseases. The ApoE2 displays defective binding to low-density lipoprotein (LDL) receptors and increases atherogenic lipoprotein levels, while ApoE4 displays normal receptor binding, but increases the total cholesterol and LDL cholesterol (LDL-C) levels predisposing carriers to CAD. In the context of MI, it has been shown that the E4 allele is associated with risk, while E2 allele is associated with protection. Furthermore, E3/E4 genotype strongly associated with the development of MI in young South African Indians.

2. Material and methods

2.1. Study groups

The study was conducted at Govind Ballabh Pant Institute of Postgraduate Medical Education and Research, New Delhi, in collaboration with CSIR-Institute of Genomics and Integrative Biology, Mall Road, New Delhi, from January 2014 to November 2015. A total of 339 subjects were recruited in the study. The subjects were differentiated into three groups, which are as follows.

1. yMI patients: 125 cases of acute MI with age <35 years.
2. oMI patients: 111 patients of acute MI with age >35 and <80 years.
3. controls: 103 healthy controls with age ≤35 years.

Controls were recruited based on the criteria that they had no evidence of CAD on history or Electrocardiogram (ECG) and had no first-degree relative having CAD and/or any other common disease. MI patients with enzyme-positive acute MI were included in the study. MI cases were defined based on the third universal definition of MI. Family history of IHD was considered when any direct blood relative (parents, siblings, and children) or grandparent or sibling of parents had any of the following: angina, MI, sudden cardiac death without obvious cause. Hypertension was defined as ≥140 mmHg systolic blood pressure (BP) or ≥90 mmHg diastolic BP on at least two occasions or current use of any antihypertensive therapy. Diabetes was diagnosed when patient had classical symptoms of diabetes plus random plasma glucose concentration ≥200 mg/dl (11.1 mmol/l) or fasting plasma glucose ≥126 mg/dl (7 mmol/l) or 2 h post-load glucose ≥ 200 mg/dl (11.1 mmol/L) during an oral glucose tolerance test or using anti-diabetic medications.

2.2. Exclusion criteria

Subjects were excluded from the control group if they had a previous history of documented CAD, peripheral vascular disease, CVD, cerebrovascular disease, suspected or proved myocarditis, or any other serious systemic illness. MI cases were excluded if they had serious systemic illness such as myocarditis, cerebrovascular accident, comatose condition, chronic kidney disease, chronic liver disease, psychiatric illness, sepsis, connective tissue disorders, or systemic inflammatory diseases. Pregnant women and women receiving oral contraceptive or hormone replacement therapy were excluded from the study. Participants were not recruited if there was inability to give consent or did not comply with the study protocol.

All the subjects underwent estimation of following metabolic, inflammatory, and prothrombotic markers: (1) metabolic markers: fasting serum insulin level, fasting serum lipid profile [i.e. total cholesterol, triglycerides (TGs), LDL-C, and high-density lipoprotein cholesterol (HDL-C)], Lp(a), apolipoprotein A1 (ApoA1), and apolipoprotein B (ApoB); (2) inflammatory markers: hsCRP and IL-6; and (3) prothrombotic markers: homocysteine and fibrinogen. All the subjects also underwent ApoE genotyping. Further details of blood sampling and various biochemical and genetic analyses are provided in Appendix A.

2.3. Statistical analysis

SPSS 16.0 (SPSS Inc., Chicago, Illinois, USA) and EPIINFO version 6 (Centers for Disease Control, Atlanta, Georgia, USA) software and Simple Interactive Statistical Analysis online tool (http://www.quantitativeskills.com/sisa/) were used for statistical analysis. The biochemical parameters were expressed as mean ± standard error. The difference between the groups was analyzed with general linear model. All the biochemical data were adjusted with age, gender, body mass index (BMI), family history of IHD, CAD history, smoking history, tobacco history, and presence of hypertension and diabetes. Age was excluded from confounding factors, when yMI patients were compared with oMI patients. A goodness of fit test was used for testing the Hardy-Weinberg equilibrium (HWE), and a χ² test compared the genotype and allele frequencies of ApoE polymorphism among the three groups. In the genetic analysis, the power of the sample size to detect the association at α = 0.05 was calculated using an online tool “OSSE-An Online Sample Size Estimator” (link: http://osse.bii.a-star.edu.sg/calculation2.php). A p value of ≤0.05 was considered statistically significant.

3. Results

3.1. Clinical characteristics

The main clinical and demographic characteristics of all the participants are presented inTable 1. There was a significant difference in gender ratio and age of yMI patients as compared with controls and oMI patients (p ≤ 0.01). OMI patients had the highest BMI among the groups. yMI patients had significantly high family history of IHD as compared with oMI patients (p = 0.01) that might be a risk factor for MI in young patients.

Angiographic parameters of MI patients in both groups, i.e., yMI and oMI, are highlighted in Supplementary Table S1. The angiographic parameters were the type of diagnosis, i.e. non–ST-elevation myocardial infarction or ST-elevation myocardial infarction, type of coronary angiography (CAG), presence of thrombus, presence of 99–100% occlusion, and presence of diffuse disease in the artery/arteries. The oMI group did not have any patient with a normal CAG as compared with the yMI group (5.6%; p = 0.03). Furthermore, significant number of patients in the oMI group (22.5%) had double-vessel disease with respect to the yMI group (12.0%; p = 0.03).

3.2. Biochemical analyses

Levels of various biomarkers studied are summarized in Table 2 and Fig. 1. Serum TG was significantly low in yMI patients than in controls (p = 0.024; Fig. 1c). Serum HDL-C was lowest in yMI
patients as compared with other two groups, i.e. controls and oMI patients (p = 2.63E-04 and p = 1.29E-05, respectively; Fig. 1e). ApoA1 level was also lowest in yMI patients, but significant only in comparison to controls (p = 2.62E-04; Fig. 1g). The yMI group had highest ratios of total cholesterol:HDL-C (p = 0.027 in yMI patients versus controls and p = 0.018 in yMI patients versus oMI patients; Fig. 2a), LDL-C:HDL-C (p = 0.002 in yMI patients versus controls and p = 0.005 in yMI patients versus oMI patients; Fig. 2b), and ApoB:ApoA1 (p = 8.75E-05 in yMI patients versus controls and p > 0.05 in yMI patients versus oMI patients; Fig. 2c). Inflammatory marker, hsCRP and prothrombotic marker, fibrinogen were significantly high in yMI patients than in controls (p = 0.001 and p = 0.02, respectively) but lower than in oMI patients (p > 0.05; Figs. 3a and 4a).

Correlation among the biomarkers showed that insulin and fibrinogen positively correlated in healthy controls (Table 3). Total cholesterol had significant positive correlation with TG, LDL-C, and ApoB in all the three groups (Tables 3-5). In addition, total cholesterol positively correlated with ApoA1 in both oMI and yMI patients and positively correlated with HDL-C in oMI patients and homocysteine in yMI patients (Tables 4 and 5). LDL-C and ApoB positively correlated in all the three groups, whereas LDL-C also positively correlated with HDL-C and ApoA1 in oMI patients and with homocysteine in yMI patients (Tables 3-5). HDL-C positively correlated with ApoA1 in controls and yMI patients while with ApoB in oMI patients. A positive correlation was observed between Lp(a) and fibrinogen in controls. ApoA1 and ApoB positively correlated in all the three groups. Moreover, ApoA1 also positively correlated with hsCRP in controls and homocysteine in oMI patients. Furthermore, ApoB positively correlated with hsCRP in controls (Table 3).

Biomarker levels were independent of angiographic parameters within yMI and oMI groups (Supplementary Table S2 and S3).

### 3.3. Genetic analyses

Genotyping was performed in 257 best-quality DNA samples, comprising 89 healthy subjects, 86 oMI patients, and 82 yMI patients.

---

**Table 1**

Clinical and demographic characteristics of studied subjects.

| Parameters                  | Study groups | P value      |
|-----------------------------|--------------|--------------|
|                             | Control (n = 103) | oMI (n = 111) | yMI (n = 125) | oMI vs control | yMI vs control | yMI vs oMI |
| **Gender**                  |              |              |              |               |               |            |
| Male                        | 60 (58%)     | 94 (85%)     | 119 (95%)    | <0.001        | <0.001        | 0.01        |
| Female                      | 43 (42%)     | 17 (15%)     | 6 (5%)       |               |               |             |
| **Clinical characteristics**|              |              |              |               |               |             |
| Age (years)                 | 27.97 ± 4.07 | 21.90 ± 6.99 | 29.33 ± 4.01 | <0.001        | 0.01          | <0.001      |
| BMI (kg/m²)                 | 22.24 ± 4.46 | 24.13 ± 4.88 | 23.06 ± 4.32 | 0.004         | NS            | NS          |
| Normal BMI                  | 80 (78%)     | 69 (62%)     | 91 (73%)     | 0.01          | NS            | NS          |
| Preobesity                  | 14 (13%)     | 24 (22%)     | 24 (19%)     | NS            | NS            | NS          |
| Class I obesity             | 9 (9%)       | 14 (13%)     | 7 (6%)       | NS            | NS            | NS          |
| Class II obesity            | 0 (0%)       | 4 (3%)       | 3 (2%)       | NS            | NS            | NS          |
| **Life style/history**      |              |              |              |               |               |             |
| Family history of IHD       | 34 (33%)     | 28 (25%)     | 52 (42%)     | NS            | NS            | 0.01        |
| Tobacco history             | 1 (1%)       | 5 (5%)       | 4 (3%)       | NS            | NS            | NS          |
| Smoking history             | 22 (21%)     | 66 (59%)     | 82 (66%)     | <0.001        | <0.001        | NS          |
| CAD history                 | 0 (0%)       | 3 (3%)       | 7 (6%)       | NS            | NS            | NS          |
| Hypertension                | 5 (5%)       | 27 (24%)     | 13 (10%)     | <0.001        | NS            | 0.002       |
| Diabetes                    | 2 (2%)       | 23 (21%)     | 6 (5%)       | <0.001        | NS            | <0.001      |

BMI, body mass index; CAD, coronary artery disease; IHD, ischemic heart disease; n, number of samples; NS, non-significant p value.

Data are represented as number of samples (percentage), except age and BMI, which are represented as mean ± standard deviation. Unpaired Student’s t-test was used to compare the data between any two groups using EPIINFO version 6. p value ≤ 0.05 was considered statistically significant.

---

**Table 2**

The studied biomarkers levels.

| Biochemical markers (units) | Study groups | P value      |
|-----------------------------|--------------|--------------|
|                             | Control (n = 103) | oMI (n = 111) | yMI (n = 125) | oMI vs control | yMI vs control | yMI vs oMI |
| **Metabolites**             |              |              |              |               |               |             |
| Insulin (µg/ml)             | 13.86 ± 1.90 | 19.10 ± 2.41 | 15.08 ± 2.52 | 0.792         | 0.683         |             |
| Total cholesterol (mg/dl)   | 162.29 ± 3.97 | 168.22 ± 4.76 | 160.38 ± 4.58 | 0.303         | 0.251         |             |
| TG (mg/dl)                  | 160.98 ± 8.60 | 161.81 ± 8.11 | 153.41 ± 6.98 | 0.024         | 0.373         |             |
| LDL-C (mg/dl)               | 82.44 ± 2.95 | 91.87 ± 3.60 | 95.07 ± 4.79 | 0.108         | 0.651         |             |
| HDL-C (mg/dl)               | 42.03 ± 1.13 | 42.62 ± 1.93 | 33.28 ± 1.17 | 2.63E-04      | 2.74E-05      |             |
| Lp(a) (mg/dl)               | 27.32 ± 3.38 | 36.67 ± 4.00 | 37.26 ± 4.06 | 0.137         | 0.438         |             |
| ApoA1 (mg/dl)               | 1.33 ± 0.03  | 1.26 ± 0.04  | 1.16 ± 0.03  | 2.62E-04      | 0.061         |             |
| ApoB (mg/dl)                | 0.82 ± 0.02  | 0.88 ± 0.03  | 0.89 ± 0.03  | 0.108         | 0.866         |             |
| Total cholesterol:HDL-C    | 4.10 ± 0.14  | 4.60 ± 0.23  | 6.06 ± 0.48  | 0.027         | 0.018         |             |
| LDL-C:HDL-C                 | 2.06 ± 0.08  | 2.45 ± 0.13  | 3.62 ± 0.32  | 0.002         | 0.005         |             |
| ApoB:ApoA1                  | 0.63 ± 0.02  | 0.76 ± 0.05  | 0.80 ± 0.03  | 8.75E-05      | 0.873         |             |
| **Inflammatory markers**    |              |              |              |               |               |             |
| hsCRP (mg/l)                | 5.66 ± 1.74  | 34.57 ± 6.36 | 23.55 ± 4.35 | 0.001         | 0.441         |             |
| IL-6 (pg/ml)                | 129.18 ± 8.95 | 129.14 ± 8.14 | 145.00 ± 8.29 | 0.386         | 0.174         |             |
| Homocysteine (µmol/l)       | 30.08 ± 2.41 | 28.34 ± 1.72 | 36.23 ± 2.61 | 0.826         | 0.096         |             |
| Fibrinogen (mg/dl)          | 302.62 ± 10.80 | 401.41 ± 15.97 | 395.56 ± 15.60 | 0.020         | 0.477         |             |

ApoA1, apolipoprotein A1; ApoB, apolipoprotein B; ANCOVA, analysis of covariance; HDL-C, high-density lipoprotein cholesterol; hsCRP, highly sensitive C-reactive protein; IL-6, interleukin-6; LDL-C low-density lipoprotein cholesterol; Lp(a), lipoprotein(a); n, number of samples; TGs, triglycerides.

Data are represented as mean ± standard error. General linear model (ANCOVA) was used to compare the biomarker levels between the two groups after adjusting with confounding factors using SPSS 16.0. p value ≤ 0.05 was considered statistically significant.
The ApoE genotype was deduced and assigned to the study subjects based on the data obtained from genotyping of Single nucleotide polymorphisms (SNPs) rs429358 and rs7412. Supplementary Table S5 shows the genotype observed of the two SNPs and resulting ApoE genotype. The goodness of fit test showed HWE harmony between observed and expected genotype frequencies of both SNPs (p > 0.05) in all the three groups. yMI patients showed similar genotypic and allelic distribution to healthy controls, which were of the same age group (Table 6). However, yMI patients had significantly higher frequency of E3/E3 genotype as compared with oMI patients indicating E3/E3 as a risk genotype for young patients p = 0.035, odds ratio (OR) = 2.46, 95% confidence interval (CI) = 1.05–5.78; Table 7). Nevertheless, it could not be significantly established at allelic level. The E3/E4 + E4/E4 genotypes were significantly dominant in oMI patients than in yMI patients (p = 0.018, OR = 0.17, 95% CI = 0.04–0.79; Table 7). However, significance could not be achieved at the E4 allelic level or when oMI patients were compared with controls at genotype or allelic level to confer E4 risk (data not presented). Because power of the study was <80%, a larger sample size would be required to correctly exemplify the association of a risk genotype/s or allele/s.

levels of metabolic markers, i.e. total cholesterol, TG, LDL-C, HDL-C, Lp(a), ApoA1, and ApoB, and ratio of total cholesterol:HDL-C, LDL-C:HDL-C, and ApoB:ApoA1 were assessed in each genotype of MI patient categories. However, no significant interaction could be observed (Supplementary Figure S1 and S2).
Furthermore, angiographic profile of oMI and yMI patients was also evaluated based on the genotype distribution and compared between oMI and yMI patients. Nevertheless, there was no difference in distribution of angiographic data in each ApoE genotype among the MI patient groups (Supplementary Table S6).

4. Discussion

MI is a multifactorial disease affected by environmental factors and genetic variations. The present study investigated the effect of ApoE gene polymorphism and nonconventional biochemical risk factors on severity of CAD in acute MI. Conventional risk factors for MI include male gender, smoking, and a family history of early CAD. In favor of previous reports, our young patients were more likely to be males and smokers as compared with other groups. Further, family history of IHD was present in 42% of yMI patients as compared with 25% in oMI patients that can be a risk factor in the young.

We observed significantly lower levels of TG in yMI patients than in controls and oMI patients. A previous study from Bangladesh reported elevated TG levels in yMI patients. Hence, TG cannot be considered as a risk factor for young patients at this point. TG is an important biomarker in CVD, but controversies exist for TG to be a risk factor. Furthermore, it was noted that lower level of HDL-C in yMI patients was a risk factor. An inverse relationship exists between the level of HDL-C and risk of CVD. However, genetically elevated levels of HDL-C does not protect against MI. ApoA1 is the main protein moiety in HDL and a stronger prognostic marker than HDL-C. Similar to HDL-C, lowest levels of ApoA1 was observed in yMI patients indicating it as a potent risk factor in addition to HDL-C.

Certain interesting findings were observed in the present study regarding the ratios of components of lipid profile. The ratios total cholesterol:HDL-C, LDL-C:HDL-C, and ApoB:ApoA1 were significantly highest for the yMI group compared with the healthy control group and oMI group. These lipid profile ratios are known as the better predictor of CVD and may identify young dyslipidemic patients who might be at risk of acute coronary event.

In addition, we also determined inflammatory and prothrombotic markers in the three study groups. We found that...
Table 3
Correlation analysis between biomarkers in the healthy control group.

| Biomarkers | Insulin | Total cholesterol | TG | LDL-C | HDL-C | Lp(a) | ApoA1 | ApoB | hsCRP | IL-6 | Homocysteine | Fibrinogen |
|------------|---------|-------------------|----|-------|-------|-------|-------|------|-------|------|-------------|-----------|
| ApoA1      | r = 0.01 | r = 0.01          |    | r = 0.04 | r = 0.11 | r = 0.01 | r = 0.13 | r = 0.02 | r = 0.17 | r = 0.09 | r = 0.45       | r = 0.10  |
| ApoB       | r = 0.09  | r = 0.22          |    | r = 0.06  | r = 0.01  | r = 0.39 | r = 0.02  | r = 0.23 | r = 0.04 | r = 0.03 | r = 0.06       | r = 0.08  |
| hsCRP      | r = -0.03 | r = -0.05         |    | r = -0.10 | r = -0.06 | r = -0.001 | r = 0.33 | r = 0.33 | r = 0.10 | r = 0.13 | r = 0.01       | r = 0.11  |
| IL-6       | r = 0.004 | r = -0.13         |    | r = -0.13 | r = -0.09 | r = -0.16 | r = 0.23 | r = -0.13 | r = -0.01 | r = 0.11 | r = 0.01       | r = 0.11  |
| Homocysteine| r = -0.02 | r = -0.12         |    | r = -0.17 | r = -0.01 | r = 0.04 | r = 0.03 | r = 0.06 | r = 0.08 | r = 0.08 | r = 0.08       | r = 0.11  |
| Fibrinogen | r = 0.29  | r = -0.09         |    | r = -0.06 | r = -0.10 | r = -0.09 | r = 0.30 | r = -0.08 | r = 0.04 | r = -0.18 | r = -0.02       | r = 0.11  |

ApoA1, apolipoprotein A1; ApoB, apolipoprotein B; LDL-C, high-density lipoprotein cholesterol; hsCRP, highly sensitive C-reactive protein; IL-6, interleukin-6; LDL-C low-density lipoprotein cholesterol; Lp(a), lipoprotein(a); p, p value; r, correlation coefficient; TGs, triglycerides.

Partial correlation was performed using SPSS 16.0. Bonferroni-corrected p value ≤ 0.05/12 = 0.004 was considered statistically significant.
Table 4  
Correlation analysis between biomarkers in the oMI group.

| Biomarkers | Insulin | Total cholesterol | TG | LDLC | HDLC | Lp(a) | ApoA1 | ApoB | hsCRP | IL-6 | Homocysteine | Fibrinogen |
|------------|---------|-------------------|----|------|------|-------|-------|------|-------|------|-------------|-----------|
| Insulin    | r — 1   | r — 0.01          | r — 0.90 | r — 0.18 | r — 0.54 | r — 1 | r — 0.07 | p — 3.69E-09 | r — 0.06 | r — 0.88 | p — 4.24E-34 | r — 0.18 | r — 0.04 | p — 0.001 | r — 0.18 | r — 0.40 | r — 0.02 | r — 0.04 | p — 7.62E-05 | r — 0.04 | r — 0.02 | r — 0.07 | r — 0.40 | r — 0.07 | p — 0.04 | p — 0.01 | r — 0.51 | p — 0.67 | p — 0.54 | r — 0.31 | r — 1 | r — 0.38 | r — 1 | r — 0.22 | r — 0.04 | r — 1 | r — 0.23 | r — 0.04 | r — 0.01 | p — 0.01 | r — 0.51 | p — 0.67 |
| ApoA1      | r — 0.09 | r — 0.89          | r — 0.47 | r — 0.80 | r — 0.30 | r — 0.06 | r — 0.52 | r — 1 | p — 0.35 | p — 1.36E-35 | p — 6.14E-07 | p — 5.83E-24 | p — 0.002 | p — 0.58 | p — 1.62E-08 | r — 0.03 | r — 0.05 | r — 0.06 | r — 0.09 | r — 0.09 | r — 0.06 | r — 0.06 | r — 0.09 | r — 0.09 | r — 0.02 | r — 1 | p — 0.79 | p — 0.60 | p — 0.61 | p — 0.38 | r — 0.36 | p — 0.39 | r — 0.13 | r — 1 | r — 0.11 | r — 0.12 | r — 0.06 | r — 0.06 | r — 0.14 | r — 0.15 | r — 0.12 | r — 0.06 | r — 0.18 | r — 0.09 | r — 1 | r — 0.12 | r — 0.14 | r — 0.27 | r — 0.004 | r — 0.04 | r — 0.03 | r — 1 | r — 0.12 | r — 0.14 | r — 0.27 | r — 0.004 | r — 0.03 | r — 1 | r — 0.12 | r — 0.14 | r — 0.27 | r — 0.004 | r — 0.03 | r — 1 | r — 0.12 | r — 0.14 | r — 0.27 | r — 0.004 | r — 0.03 | r — 1 | r — 0.12 | r — 0.14 | r — 0.27 | r — 0.004 | r — 0.03 | r — 1 | r — 0.12 | r — 0.14 | r — 0.27 | r — 0.004 | r — 0.03 | r — 1 | r — 0.12 | r — 0.14 | r — 0.27 | r — 0.004 | r — 0.03 | r — 1 | r — 0.12 | r — 0.14 | r — 0.27 | r — 0.004 | r — 0.03 | r — 1 | r — 0.12 | r — 0.14 | r — 0.27 | r — 0.004 | r — 0.03 | r — 1 | r — 0.12 | r — 0.14 | r — 0.27 | r — 0.004 | r — 0.03 | r — 1 |

ApoA1, apolipoprotein A1; ApoB, apolipoprotein B; HDL-C, high-density lipoprotein cholesterol; hsCRP, highly sensitive C-reactive protein; IL-6, interleukin-6; LDL-C, low-density lipoprotein cholesterol; Lp(a), lipoprotein(a); oMI, old myocardial infarction; p, p value; r, correlation coefficient; TGs, triglycerides.

Partial correlation was performed using SPSS 16.0. Bonferroni-corrected p value ≤ 0.05/12 = 0.004 was considered statistically significant.
### Table 5
Correlation analysis between biomarkers in the yMI group.

| Biomarkers     | Insulin | Total cholesterol | TG       | LDL-C | HDL-C | Lp(a) | ApoA1 | ApoB | IL-6 | Homocysteine | Fibrinogen |
|----------------|---------|-------------------|----------|-------|-------|-------|-------|------|-----|--------------|------------|
| **Insulin**    | r = -0.03 | r = 1             | p = 0.97 | r = 0.05 | r = 0.64 | r = 1 |       |      |     |              |            |
| **Total Cholesterol** | r = 0.06 | r = 1             | p = 0.01 | r = 0.05 | r = 0.64 | r = 1 |       |      |     |              |            |
| **TG**         | r = -0.08 | r = -0.09         | r = 0.30 | r = -0.01 | r = 0.15 | r = 1 |       |      |     |              |            |
| **LDL-C**      | r = -0.01 | r = -0.13         | r = -0.13 | r = -0.03 | r = -0.04 | r = 1 |       |      |     |              |            |
| **HDL-C**      | r = -0.01 | r = -0.09         | r = 0.76 | r = -0.09 | r = -0.17 | r = -0.04 | r = -0.02 | r = 0.16 | r = -0.06 |                    |
| **ApoA1**      | r = -0.28 | r = 0.15          | r = 0.15 | r = 0.07 | r = 0.34 | r = 1 |       |      |     |              |            |
| **ApoB**       | r = -0.01 | r = -0.13         | r = -0.13 | r = -0.01 | r = -0.04 | r = 1 |       |      |     |              |            |
| **hsCRP**      | r = -0.01 | r = -0.03         | r = -0.03 | r = -0.01 | r = -0.09 | r = 1 |       |      |     |              |            |
| **IL-6**       | r = -0.01 | r = -0.03         | r = -0.03 | r = -0.01 | r = -0.09 | r = 1 |       |      |     |              |            |
| **Homocysteine** | r = -0.01 | r = -0.03         | r = -0.03 | r = -0.01 | r = -0.09 | r = 1 |       |      |     |              |            |
| **Fibrinogen** | r = -0.01 | r = -0.03         | r = -0.03 | r = -0.01 | r = -0.09 | r = 1 |       |      |     |              |            |

Ap0A1, apolipoprotein A1; ApoB, apolipoprotein B; HDL-C, high-density lipoprotein cholesterol; hsCRP, highly sensitive C-reactive protein; IL-6, interleukin-6; LDL-C, low-density lipoprotein cholesterol; Lp(a), lipoprotein(a); p, p value; r, correlation coefficient; TGs, triglycerides; yMI, young myocardial infarction.

Partial correlation was performed using SPSS 16.0. Bonferroni-corrected p value ≤ 0.05/12 = 0.004 was considered statistically significant.
similar to oMI patients, yMI patients had increased levels of hsCRP and fibrinogen than the controls, but the levels were lower than oMI patients. This observation suggests that hsCRP and fibrinogen can be a risk factor for both young and old patients, but precision is high in the elderly. Interestingly, higher baseline CRP levels were observed in control population. Kamath et al23 reported that in healthy Indians, the hsCRP levels ranges from intermediate to high risk level, indicating that the basal concentration of hsCRP is high in healthy Indians. Also, Indians living in western countries have higher hsCRP levels compared with native population. However, this needs to be confirmed in further studies.

Further, this study also reports interesting correlations among the studied biomarkers. Among several observed correlations, the positive correlations between fibrinogen and insulin and between fibrinogen and Lp(A) in the healthy controls seem interesting. The relationship between fibrinogen and insulin resistance in diabetic or nondiabetic patients have been reported earlier.24–26 Because insulin resistance is normal in controls, a casual correlation might exist between fibrinogen and insulin levels in our study; however, further evaluation is required. Fibrinogen is essential for the formation of blood clots; hence, it is positively correlated to Lp(A), which contributes to the process of thrombogenesis and atherogenesis. However, such correlation was not observed in oMI or yMI patients, which have increased levels of fibrinogen and Lp(A). The observation suggests that the elevation in the levels of fibrinogen and Lp(A) in MI patients is independent of each other.

In the process of differentiating yMI and oMI patients, we observed positive correlations of homocysteine with total cholesterol, TGs, and LDL-C in yMI patients. Controversies exist in considering homocysteine as a risk factor for CVD; however, it is an independent risk factor for atherosclerosis.27–30 Thus, a relationship is apparent between homocysteine and lipid profile. The correlation of homocysteine with total cholesterol, TGs, and LDL-C might be a unique feature for yMI patients. However, one cannot deny the possibility of the presence of such correlations in the elderly. In an atherosclerosis cohort of old patients (≥40 years), homocysteine correlates with the increase in TG and decrease in HDL-C.31 Most of the oMI patients are under the treatment of statins that decrease total cholesterol, TGs, and LDL-C, increase HDL-C, and have no effect on homocysteine. Therefore, this correlation may not be observed in oMI patients. Further, studies are required to rule out the possible effect of statin treatment and to affirm that the observed correlations in yMI patients are their unique feature.

The present study also investigated the genetic role of ApoE polymorphism in yMI and oMI patients. ApoE aids in the transport of lipoproteins and metabolism of fats. The involvement of ApoE in CAD was known since 1980s.32,33 However, how different polymorphism of ApoE might predispose young patients to MI is still unclear. Meta-analysis on the risk of MI and coronary heart disease has revealed that the E4 allele is associated with the increased risk, while E2 allele had a decreased risk.34,35 The aim of the genetic investigation was to determine if any difference exists in yMI and oMI patients with respect to E2, E3, and E4 alleles of ApoE. No strong conclusions could be made from the present study because of the small sample size and low statistical power. A larger sample size could establish whether yMI patients have a high frequency of any particular ApoE allele that may act as a risk allele.

5. Conclusion

The present study investigated the role of nonconventional biochemical risk factors in MI in very young patients. Most importantly, low HDL-C and ApoA1 levels are possible risk markers for MI at young age. The present study for the first time reports that young MI patients have significantly higher ratios of total
cholesterol:HDL-C, LDL-C:HDL-C, and ApoB:ApoA1 than healthy controls and oMI patients. These increased ratios are also the risk factors for yMI patients along with HDL-C and ApoA1.

ApoE gene polymorphism did not show any significant association with MI in very young patients. Further studies are required to affirm ApoE allele risk for MI in young and thus further define the young cohort.

Funding

This work was supported by the Council of Scientific and Industrial Research, India via project BSC0123.

Key messages

- What is already known about this subject?
  - ApoE3/E4 genotype strongly associated with the development of MI in young South Asian Indians. It has been shown that the E4 allele is associated with MI risk, while E2 allele is associated with MI protection.
  - However, angiographic data and biochemical and novel risk marker profile in very young patients with MI is limited. In fact, there is no study from Southeast Asia that has studied these parameters.

- What does this study add?
  - This is the first study to analyze biochemical and genetic profile of yMI patients (<35 years).
  - ApoE gene shows no significant association with MI at young age.
  - Low levels of serum HDL-C and ApoA1 are possible risk markers for MI at young age.
  - Very yMI patients have higher ratio of total cholesterol:LDL-C, LDL-C:HDL-C, and ApoB:ApoA1 than oMI patients and the controls.

- How might this impact on clinical practice?
  - This is the first study which is bringing the fact in light that very yMI patients have significantly higher ratios of total cholesterol:HDL-C, LDL-C:HDL-C, and ApoB:ApoA1 than healthy controls and oMI patients. These parameters may be predictive of future development of ACS in these patients.

Conflict of interest

All others have none to declare.

Acknowledgments

We thank all the subjects who participated in the study.

Appendix B. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ihj.2018.08.013.

Appendix. A

Materials and methods

Sample collection

Subjects with overnight fasting were rested for 30 min before drawing blood in supine position. Five to eight milliliter of blood was drawn in acid citrate dextrose anticoagulant. Two to three milliliter blood was transferred to another vial and clotted to collect serum for estimation of biochemical parameters. The remaining blood sample was centrifuged (A-4–62, Eppendorf, Hamburg, Germany) for 10 min at 1500 rpm at 4 °C for plasma extraction. The latter was also used for the analyses of biochemical parameters. Peripheral blood leucocytes were used for DNA extraction by modified salting-out procedure reported by Miller et al, 1988.

Biomarker estimation

The study included metabolic, inflammatory, and prothrombotic markers to characterize yMI cases. The following nontraditional biochemical factors were assessed: (1) metabolic markers: fasting serum insulin level, fasting serum lipid profile (i.e. total cholesterol, triglycerides (TGs), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), Lp(a), apolipoprotein A1 (ApoA1) and apolipoprotein B (ApoB)); (2) inflammatory markers: hsCRP and IL-6; and (3) prothrombotic markers: homocysteine and fibrinogen.

Serum insulin was determined by Cobas e 411 Elecsys modular (Roche Diagnostics GmbH, Mannheim, Germany), using electrochemiluminescence immuno assay (Roche Diagnostics GmbH). Total cholesterol, TG, LDL-C, and HDL-C were estimated by enzymatic colorimetry kit (Roche Diagnostics GmbH) and Lp(a), ApoA1, ApoB, and hsCRP were estimated by using immunoturbidimetric assay (Roche Diagnostics GmbH) on Cobas c501 autoanalyzer (Roche Diagnostics GmbH). Levels of IL-6 (Diaclone SAS, Besancon, France) and homocysteine (Roche Diagnostics GmbH) were determined by enzyme-linked immunosorbent assay on Cobas c501 autoanalyzer (Roche Diagnostics GmbH). Serum fibrinogen was estimated by immunoturbidimetric kit from Instrumentation Laboratory on Elite pro automated analyzer (Instrumentation Laboratory, Orangeburg, New York, USA).

Genotyping

The ApoE genotype was deduced and assigned to the study subjects based on the data obtained from SNaPshot genotyping of SNPs rs429358 and rs7412. The ApoE loci of 352bp consisting of SNPs rs429358 and rs7412 was amplified by polymerase chain reaction (PCR) method using forward, 5′-CTA CAA ATC ATC GAG ACT GGA GG -3′ and reverse, 5′-CAC GCC CCG CTG TTC CAC GAG-3′ primers. The 20 µl of reaction mixture contained 50 ng of DNA, 3 pmol of each primer, 1× buffer, 0.33U of Taq DNA polymerase, and 0.2 mmol/l of deoxynucleotide triphosphates. The PCR cycling condition was 95 °C initial denaturation for 5 min, followed by 38 cycles of 95 °C denaturation for 40 s, 56 °C annealing for 30 s, 72 °C extension for 45 s, and 72 °C final extension of 10 min. The PCR product was purified by polyethylene glycol purification and was subjected to genotyping by using a SNaPshot multiplex kit (Applied Biosystems, Foster City, California, USA). The SNaPshot primer sequences for rs429358 and rs7412 were 5′-GCC GGA CAT GGA GGA GTT ATC GGA GG -3′ (annealing at 56 °C) and 5′-ATG CCG CAC ATT CTG TAC TGA GGA GGA GGA -3′ (annealing at 57 °C), respectively. All the primers were designed by PerlPrimer software. Peak Scanner Software v1.0 was used for allele determination.

References

1. Lopez AD, Mathers CD, Ezzati M, et al. Global and regional burden of disease and risk factors, 2001: systematic analysis of population health data. Lancet. 2006;367(9524):1747–1757.
2. Xavier D, Pais P, Devereaux PJ, et al. Treatment and outcomes of acute coronary syndromes in India (CREATE): a prospective analysis of registry data. Lancet. 2008;371(9622):1435–1442.
3. Pineda J, Marín F, Marco P, et al. Premature coronary artery disease in young (age <45) subjects: interactions of lipid profile, thrombophilic and haemostatic markers. Int J Cardiol. 2009;136(2):222–225.

4. Mahley RW. Apolipoprotein E: from cardiovascular disease to neurodegenerative disorders. J Mol Med (Berl). 2016;94:739–746.

5. Wang YL, Sun LM, Zhang L, et al. Association between Apolipoprotein E polymorphism and myocardial infarction risk: a systematic review and meta-analysis. FEBS Open Biol. 2015;5:852–858.

6. Ranjith N, Pegoraro RJ, Rom L, et al. Lp(a) and ApoE polymorphisms in young African Indians with myocardial infarction. Cardiovasc J South Afr. 2004;15(3):111–117.

7. Thygesen K, Alpert JS, Jaffe AS, et al. Third universal definition of myocardial infarction. Circulation. 2012;126(16):2020–2035.

8. James PA, Oparil S, Carter BL, et al. 2014 evidence-based guideline for the management of high blood pressure in adults: report from the panel members appointed to the Eighth Joint National Committee (JNC 8). JAMA. 2014;311(5):507–520.

9. American Diabetes Association. (2) Classification and diagnosis of diabetes. Diabetes Care. 2015;38(suppl):S8–S16.

10. Egiziano G, Akhtari S, Pilote L, et al. Sex differences in young patients with acute myocardial infarction. Diabet Med. 2013;30(3):e108–e114.

11. Yunyun W, Tong L, Yingwu L, et al. Analysis of risk factors of ST-segment elevation myocardial infarction in young patients. BMC Cardiovasc Disord. 2014;14:179.

12. Colkesen AY, Acil T, Demircan S, et al. Coronary lesion type, location, and characteristics of acute ST elevation myocardial infarction in young adults under 35 years of age. Coron Artery Dis. 2008;19(5):345–347.

13. Gaeta G, De Michele M, Cuomo S, et al. Arterial abnormalities in the offspring of patients with premature myocardial infarction. N Engl J Med. 2000;343(12):840–846.

14. Karim MA, Majumder AA, Islam KQ, et al. Risk factors and in-hospital outcome of acute ST segment elevation myocardial infarction in young Bangladeshi adults. BMC Cardiovasc Disord. 2015;15:73.

15. Miller M, Stone NJ, Ballantyne C, et al. Triglycerides and cardiovascular disease: a scientific statement from the American Heart Association. Circulation. 2011;123(20):2292–2333.

16. Sarwar N, Danesh J, Eiriksdottir G, et al. Triglycerides and the risk of coronary heart disease: 10,158 incident cases among 262,525 participants in 29 Western prospective studies. Circulation. 2007;115(4):450–458.

17. Austin MA, Hokanson JE, Edwards KL. Hypertriglyceridemia as a cardiovascular risk factor. Am J Cardiol. 1998 Feb 26;81(4A):78–128.

18. Rader DJ, Hovingh GK. HDL and cardiovascular disease. Lancet. 2014;384(9943):618–625.

19. Hewing B, Moore KJ, Fisher EA. HDL and cardiovascular risk: time to call the plumber? Circ Res. 2012;111(9):1117–1120.

20. Voigt BF, Peluso GM, Orho-Melander M, et al. Plasma HDL cholesterol and risk of myocardial infarction: a mendelian randomisation study. Lancet. 2012;380(9841):572–580.

21. Wen J, Zhong Y, Kuang C, et al. Lipoprotein ratios are better than conventional lipid parameters in predicting arterial stiffness in young men. J Clin Hypertens (Greenwich). 2017;19(8):771–776.

22. Arsenault BJ, Boekholdt SM, Kastelein JJ. Lipid parameters for measuring risk of cardiovascular disease. Nat Rev Cardiol. 2011;8(4):197–206.

23. Kamath DY, Xavier D, Segalani A, Pais P. High sensitivity C-reactive protein (hsCRP) & cardiovascular disease: an Indian perspective. Indian J Med Res. 2015;142:261–268.

24. Martins C, Mazza do Nascimento M, Pecoits-Filho R, et al. Insulin resistance is associated with circulating fibrinogen levels in nondiabetic patients receiving peritoneal dialysis. J Ren Nutr. 2007;17(2):132–137.

25. Barazzoni R, Kiwanuka E, Zanetti M, et al. Insulin acutely increases fibrinogen production in individuals with type 2 diabetes but not in individuals without diabetes. Diabetologia. 2003;46(7):1851–1856.

26. Raynaud E, Pérez-Martín A, Brun J, et al. Relationships between fibrinogen and insulin resistance. Atherosclerosis. 2000;150(2):365–370.

27. Ganguly P, Alam SF. Role of homocysteine in the development of cardiovascular disease. Nutr J. 2015;14:6.

28. McCully KS. Homocysteine metabolism, atherosclerosis, and diseases of aging. Compr Physiol. 2015;6(1):471–505.

29. Pang X, Liu J, Zhao J, et al. Homocysteine induces the expression of C-reactive protein via NMDA-R-ROS-MAPK-NF-κB signal pathway in rat vascular smooth muscle cells. Atherosclerosis. 2014;236(1):73–81.

30. Faeh D, Chiofalo A, Paccaud F. Homocysteine as a risk factor for cardiovascular disease: should we (still) worry about? Swiss Med Wildy. 2006;136(47–48):745–756.

31. Momim M, Jia J, Fan F, et al. Relationship between plasma homocysteine level and lipid profiles in a community-based Chinese population. Lipids Health Dis. 2017;16:54.

32. Menzel HJ, Kladetzky RG, Assmann G. Apolipoprotein E polymorphism and coronary artery disease. Arterioscler Thromb Vasc Biol. 2003;23(4):310–315.

33. Davignon J, Gregg RE, Sing CF. Apolipoprotein E polymorphism and atherosclerosis. Arterioscler Thromb Vasc Biol. 1988;8(1):1–21.

34. Xu M, Zhao J, Zhang Y, et al. Apolipoprotein E gene variants and risk of myocardial infarction in young Bangladeshi adults. BMC Cardiovasc Disord. 2015;15:73.

35. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucl Acids Res. 1988;16(3):1215.