ORIGINAL ARTICLE

Preliminary Coronary Artery Disease (CAD) Case and Control Study of Total Homocysteine (Hcy), Clinico-laboratory and Sociodemographic Profiling Among Respondents in Klang Valley, Malaysia

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ABSTRACT

Introduction: Coronary artery disease (CAD) is a multifactorial pathogenesis ailment usually associated with hypertension, hyperlipidemia and dysregulated biochemical characteristics. **Materials and methods:** This case-control study analysed risk factors such as age, gender, ethnicity, BMI, SBP and DBP which have been linked to CAD. TC, TG, HDL, LDL, smoking status, exercise habit, obesity, family history of CVD and tHcy were also assessed to elucidate their association with CAD. Seventy-four respondents were diagnosed as CAD patients with angiography assessment at National Health Institute (NHI) Malaysia. Three hundreds and ten healthy respondents with no CAD signs or history were recruited from NHI, UPM and National Blood Bank (NBB). This study explores sociodemographic and clinico laboratory risk factors which contribute to the CAD conditions. **Results:** Individuals with CAD had an average age of 53.66 ± 7.904 years old, significantly different with non-CAD respondents average age (32.07 ± 10.080), $p<0.05$. Malays were found to be the highest prevalent ethnic of having CAD, followed by Indians and Chinese,with p-value <0.05. Respondents with CAD had significantly greater BMI (29.11 ± 3.660) kg/m^2 and higher SBP (135.16 ± 7.406) mmHg with $p<0.05$ compared to non-CAD. Laboratory results for respondents with CAD displayed higher TC (5.025 ± 1.009) mmol/L, TG (1.696 ± 0.222) mmol/L, LDL (3.483 ± 0.350) mmol/L and total Hcy levels (9.807 ± 2.149) µmol/L with p-value<0.05. **Conclusion:** Current preliminary findings ascertained that respondents with CAD were associated withthe risk of age , ethnicity and biochemical characteristics that could be further investigated for future study.
Malaysian Journal of Medicine and Health Sciences (2024) 20(SUPP11): 57-64. doi:10.47836/mjmhs20.s11.9

Keywords: Case-control study, Coronary artery disease, Cardiovascular, Homocysteine, Sociodemographics, Biochemical characteristics

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INTRODUCTION

The prevalence of coronary artery disease (CAD), has increased yearly not only in Malaysia but also worldwide (1). CAD causes considerable morbidity and mortality, accounting for over 1 million annual hospitalizations globally. Two primary causes that have been established as the major risk factors of CAD are the non-modifiable factors namely familial diseases influenced by genetic mutations as well as the modifiable factors present as the effect of the environment and individual lifestyle (2). By detailing these factors, suggestions for the development of therapeutic strategies and, more importantly, preventive strategies in terms of lifestyle changes as well as medical interventions and therapies can be proposed to the public and policymakers.

Cardiovascular disease (CVD)-related modifiable risk factors include sedentary behaviour, tobacco use and unhealthy dietary intake. In contrast, certain non-modifiable risk factors namely; family history to CVD, age, gender, and ethnicity have been reported to increase the likelihood of developing CVD as well. Longitudinal and retrospective studies have demonstrated that sociodemographic and biochemical parameters, including fasting blood glucose (FBG), lipid profiles (including total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL), and high density lipoprotein (HDL), significantly contribute to the development of CVD risk (3,4).

Hyperhomocystein (Hcy) is a condition whereby the higher level of homocysteines than normal. Although, Hcy has been linked to CVD for the last 50 years (5,6,7), hyperhomocystenemia is still not considered as a conclusive, mainstream risk factor for CVD such as hyperlipidemia. Among the reason why Hcy is not considered as such is because of its notable level variations in different regions (8) where the cut-off hyperhomocysteine level is between 10 – 15 µmol/L (9). The CAD respondents also believed to have a significant prevalence of vitamin B complex and folate deficiency (10). Additionally, the interactions between those who prescibed medications such as statins and atorvastatins for CVD could alter the Hcy levels (11). There are three groups of homocysteine levels based on range such as moderate (16 to 30 micromol/L), intermediate (31 to 100 micromol/L), and severe which was above 100 micromol/L) (Morris et al., 2017) (12). Nevertheless, the majority of studies have found that elevated Hcy is associated with an increased risk of CVD (13,14). According to a recent meta-analysis, the risk of CVD was elevated by 10% for each 25% increased in plasma Hcy levels (15). For this reason, the present study is carried out to identify the influence of Hcy towards CVD in a Malaysian study population.

Hcy, a sulfur-containing amino acid, is an intermediate precursor physiologically critical for cell cycle progression and maintenance of cellular homeostasis (16). Hcy is generated from the metabolism of methionine which is metabolized either by the remethylation process or by the transulfuration pathway (17,18). This may be explained by the dysregulated homocysteine levels which may in turn initiate CVD progression through blood coagulant disorders and oxidative stress-inducing injuries of vascular endothelium and arterial walls (19,20).

Our previous preliminary study, Ismil et al. (14) discovered that the female and the Chinese in a Malaysian healthy cohort study had exhibited a higher prevalence of genotypic homozygous TT and CT thermolabile of both *methylenetetrahydrofolate reductase (MTHFR)* and *cytoplasm serine hydroxymethyl transferase (cSHMT)* genes polymorphisms which were the strong genetic risk in CVD (14). In an effort to gain more conclusive data to support the idea that these genes polymorphisms may be associated with the risk of developing CAD, current investigation explores sociodemographic and clinico laboratory risk factors which may contribute to this condition. This data is important to establish the foundation of a more intense analysis to associate and conclude all possible CVD risk factors. CVD is caused by multifactorial confounding factors. Hcy level for example has been implied as one of the relevant factors that could influence CVD onset. This study is hoped to gain clearer insight on the CVD risk factors so that suitable, more precise preventive measures can be recommended.

MATERIALS AND METHODS

Respondents recruitment

The respondents were divided into two groups: cases and controls. Cases were defined as individuals with a confirmed diagnosis of coronary artery disease (CAD), while controls were those without CAD. The recruitment of the respondents upon written consent started from January and end in December 2012. Sample size is determined by using the formula suggested by Elashoff & Lemeshow (21) and Kawamoto et al.(22).

1. CAD group : 97 (where 50% frequency stroke patients as case subject)

$$\frac{50}{100} \quad X\ 97 \quad = 48.5 \sim 49$$

2. Non-CAD group : 241 (where 35% frequency in healthy control subjects)

$$\frac{35}{100} \quad X\ 241 \quad = 84.35 \sim 84$$

Based on the inclusion and exclusion criteria for cases and controls, 310 healthy respondents were gathered from the National Heart Institute, Malaysia (NHI), Universiti Putra Malaysia (UPM), and the National Blood Bank, Malaysia (NBB). The inclusion criteria was regardless gender, ethnicity mainly Malay, Chinese and Indian, and the aged was between 18 and 65 years old. The respondents were indentified no CVD and stated healthy when the recruitment process ongoing. Meanwhile, the exclusion criteria for both cases and controls were pregnant women, people under 18 years old, people with transmitted diseases, and people suffering from chronic diseases such as hypertension, renal failure, and diabetes. Seventy-four CAD patients recruited for the case group must be diagnosed and confirmed having CAD by angiography examination at the NHI by the specialist ,. Respondents in both groups of non-CAD and CAD were asked to fast for at least 12-hour before the day of blood collection . On the day of recruitment, they were informed about the study, given a consent form to sign and respondent background information sheets to complete before medical officer collected 10 mL of fasting blood.. The blood sample in ethylenediaminetetraacetic acid (EDTA) tubes was stored at 4°C. The plasma was separated beforehand by centrifugation at 9000 x g for 15 min. The separated plasma was transferred out and stored in a 5 mL normal tube in a -80 °C for biochemical analysis.

The National Medical Research Registry (NMRR) of the Malaysian Ministry of Health and the UPM Ethics Committee both approved this study, which is registered there under the identification number NMRR-08-1104-2259.

In addition to personal cardiovascular event history and family history of CVD, the following vascular risk factors were recorded, body mass index (BMI), systolic blood pressure (SBP), diastolic blood pressure (DBP). Next, total plasma cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL) and low density lipoprotein(LDL) levels were determined using Roche/Hitachi 902 (Hitachi High-Technologies Corporation, Japan) analyzer. Total homocysteine (tHcy) from fasting in unheparinized plasma was measured by using the Roche/Elecsys 2010 analyzer (Hitachi High-Technologies Corporation, Japan).

Statistical analysis
The data was analyzed using SPSS version 27. Normality test was applied in order to elucidate the distribution of the data for each of the variables. Results were presented as means and standard deviation (SD) or proportions (%). Differences between means were assessed by t-test for unmatched samples, or by non-parametric methods when the normality tested skewened. The Chi-square (χ^2) test and the independent sample t-test were used to compare the sociodemographic and biochemical characteristics of the study population. Covariables like age, gender, race, smoking status, exercise frequency, SBP, DBP, BMI, abdominal obesity, and lipid profiles (TC, TG, HDL, LDL and total tHcy) were used in multivariate analyses. A *p*-value of less than 0.05 was considered as significant.

Ethical statement
This research was funded by Research University Grant Scheme (RUGS) 04-02-07-0433 RU of Universiti Putra Malaysia.

RESULTS

A total of 384 respondents including 209 males and 175 females with a mean age of 42.865 ± 8.992 year-old were recruited for analysis. The sociodemographic and clinico laboratory data were summarised in Table I. The respondents were divided into two groups namely case and control based on the interview, as well as their predisposition exclusion and inclusion criteria that were initially set.

Table I: Comparison between CAD and non-CAD respondents on socio-demographic and clinical profiling.

Profiles	Group		χ^2	p-value	OR	95% CI	TOTAL
	CAD N=74 (%) or Mean ± SD	Non-CAD N=310 (%) or Mean ± SD					
SOCIODEMOGRAPHIC CHARACTERISTICS							
Age	53.66 ± 7.904	32.07 ± 10.080	171.328				
≥ 45 years	65 (62.5)	39 (37.5)	NA	**0.000***	50.185	23.151-108.789	104
< 45 years	9 (3.21)	271 (96.79)	NA				280
Gender							
Male	46 (22.01)	163 (77.99)	2.211	0.137	1.482	0.881-2.492	209
Female	28 (16)	147 (84)					175
Ethnicity							
Chinese	12 (9.76)	111 (90.24)	-	-	-	-	123
Malay	35 (25.36)	103 (74.64)	10.728	**0.001***	3.143	1.548-6.383	138
Indians	27 (21.95)	96 (78.05)	6.856	**0.009***	2.604	0.185-0.800	123
Smokers							
Current smokers	31 (32.63)	64 (67.37)	14.484	**0.000***	2.771	1.619-4.783	95
Non-smokers	43 (14.88)	246 (85.12)					289
Exercise							
Yes	27 (24.55)	83 (75.45)	2.757	0.097	0.636	0.372-1.088	110
No	47 (17.15)	227 (82.85)					274
Family history of CVD							
Yes	46 (32.17)	97 (67.83)	24.361	**0.000***	3.608	2.129-6.114	143
No	28 (11.62)	213 (88.38)					241

CONTINUE

Table I: Comparison between CAD and non-CAD respondents on socio-demographic and clinical profiling. (CONT.)

Profiles	Group		χ^2	p-value	OR	95% CI	TOTAL
	CAD N=74 (%) or Mean ± SD	Non-CAD N=310 (%) or Mean ± SD					
CLINICO LABORATORY							
BMI (kg/m²)	29.11 ± 3.660	25.51 ± 5.020	NA	**0.000***	NA	NA	NA
Systolic blood pressure (mmHg)	135.16 ± 7.406	125.65 ± 11.78	NA	**0.000***	NA	NA	NA
Diastolic blood pressure (mmHg)	80.16 ± 4.236	78.80 ± 8.41	NA	0.212	NA	NA	NA
Abdominal obesity							
Yes	36 (48)	39 (52)	24.361	**0.000***	6.583	3.737 - 11.595	75
No	38 (18.18)	271 (81.82)					209
TC (mmol/L)	5.025 ± 1.009	4.51 ± 0.860	NA	**0.000***	NA	NA	NA
High	34 (35.05)	63 (64.95)	20.775	**0.000***	3.333	1.953 - 5.687	97
Normal	40 (13.94)	247 (86.06)					287
TG (mmol/L)	1.696 ± 0.222	1.54 ± 0.600	NA	**0.039***	NA	NA	NA
High	32 (27.12)	86 (72.88)	6.744	**0.009***	1.984	1.177 - 3.347	118
Normal	42 (15.79)	224 (84.21)					266
HDL (mmol/L)	1.154 ± 0.219	1.25 ± 0.300	NA	**0.010***	NA	NA	
Low	11 (18.97)	47 (81.03)	0.004	0.949	0.977	0.480-1.991	58
Normal	63 (19.33)	263 (80.67)					326
LDL (mmol/L)	3.483 ± 0.350	2.77 ± 0.550	NA	**0.000***	NA	NA	NA
High	37 (46.84)	42 (53.16)	47.280	**0.000***	6.209	3.554-10.849	79
Normal	37 (12.17)	267 (87.83)					304
Total homocysteine (tHcy) (mmol/L)	**9.807 ± 2.149**	**7.183 ± 1.852**	NA	**0.000***	NA	NA	NA
High	29 (53.70)	25 (46.30)	47.888	**0.000***	7.347	3.950-13.663	54
Normal	45 (13.64)	285 (86.36)					330

Data were analyzed using contingency χ^2 test and the result showed the odds ratio value and the 95% CI value. Statistically significance values (at p<0.05) are shown in asterisk (*).χ^2 = Chi square; OR = Odds ratio; CI = confidence interval.

The characteristics of the population showed that the respondents were in majority of age below 45 year-old (73%) with $p<0.05$. From 384 respondents, there were 209 males with 46 having CAD. On the other hand, there were 175 females with 28 having CAD. Malays were the main respondents and they exhibited a significant difference in number between those having CAD or otherwise ($p<0.05$). One hundred and twenty-three Indian respondents also showed a significant difference in the number of those having CAD to those that were healthy ($p<0.05$). However, in Chinese ethnicity, there was no significant difference in the number between CAD and non-CAD groups.

Smoking status was found to be more prevalent among the non-CAD group (67.37%) compared to CAD (32.63%) with ($p<0.05$). There were no significant statistical difference in the number of respondents who exercise regularly between CAD and non-CAD groups. Also, the factor of having CVD history in the family was found to be significantly higher in non-CAD group. Also, family history of CVD was found significantly higher in non-CAD group (68%) compared to CAD group (32%). The clinico laboratory data in the present project have illuminated their importance by displaying some association with CAD. Significant differences were seen between CAD and non-CAD groups for several characteristics such as BMI (29.11 vs 25.51 kg/m2; $p < 0.05$), SBP (135.16 vs 125.65 mmHg; $p < 0.05$) and DBP (80.16 vs 78.80 mmHg, $p < 0.05$). BMI results showed that respondents in both CAD and non-CAD groups were in the overweight category. However, in the non-CAD group, BMI reading were mostly at the baseline which is in average at 25.51 ± 5.020 kg/m2. On the other hand, the respondents in the CAD group were mostly having BMI reading at 29.11 ± 3.660 kg/m2. Meanwhile, 52% respondents in non-CAD group had significantly greater an abdominal obesity compared to 48% respondents in CAD group with $p<0.05$.

An average total cholesterol level showed significantly higher in CAD compared to non-CAD groups (5.025 ± 1.009 vs 4.51 ± 0.860 mmol/L; $p<0.05$). Triglycerides (TGs) also showed significantly higher readings in CAD group (1.696 ± 0.222) mmol/L compared to non-CAD group (1.54 ± 0.600) mmol/L with p-value of <0.05. However, HDL level showed significantly lower in CAD (1.154 mmol/L ± 0.219) compared to non-CAD (1.25 mmol/L ± 0.300) groups with significant p-value of <0.05. On the other hand, LDL level showed significantly higher in CAD group (3.483 ± 0.350) mmol/L compared to non-CAD group (2.77 ± 0.550) mmol/L ($p<0.05$). Average total Hcy level was significantly higher in CAD group (9.807 ± 2.149 µmol/L) compared to non-CAD group (7.183 ± 1.852 µmol/L).

DISCUSSION

Cardiovascular diseases (CVDs) are catastrophic disorders that affect patients various physical and emotional symptoms which in turn results in high hospitalization and mortality rate thus, imposing increasing costs on society. CVD is a broad umbrella term for all types of diseases related to the heart and blood vessels. It includes CAD, which then can lead to stroke, heart failure and peripheral artery diseases (23). Medical fraternity is consistently intrigued by discovery reports of newly identified biomarkers for CAD risk. In this study, however, several conservative sociodemographic and clinico laboratory risk factors were analysed on the selected regions in Malaysia, concentrated in a few organizations situated in Putrajaya, Selangor and Kuala Lumpur. The variation in these parameters associated with CVD across different regions and countries can be attributed to a multifactorial concerns such as dietary pattern, lifestyle choice, disparities in healthcare infrastructure, genetic predisposition and socio-economic status (24).

One of the most apparent parameters in this study, is the age factor. Sixty-three percent of the respondents in the age group of 45 year-old and above were having CAD. Average age of CVD patients is 54 years old. This finding is in line with the study reported by Yazdanyar et al. (2009) who stated that ageing among elderly people would simultaneouly put them at high risk of developing CVD (25). Aging is associated with a progressive decline in numerous physiological processes. Both the heart and vasculature undergo numerous alterations during aging as a result of deregulation of molecular longevity pathways, leading to compromised function. Aging plays a vital role in the deterioration of cardiovascular functionality due to accumulation of oxidative stress, inflammation, apoptosis and myocardial degeneration throughout the years (26,27). Modifiable factors such as persistent unhealthy lifestyle could amplify the risk of CVD. This opinion is shared by Yusuf et al. (2) when they stated that smart-targeting certain modifiable risk factors according to region-specific needs is imperative because risks factors vary between countries at different economic levels for instance. This highlights the need for additional context specific priorities and more specific region studies before successful prevention measures of CVD can be carried out.

Furthermore, the current study showed that Malays (25%) are the highest respondents with CAD, followed by the Indians (22%) displayed significant of having CAD risk compared to Chinese (10%) respondents respectively. This current study corroborates a previous study reported from Singapore, which affirmed that there are significant differences in mortality from CVD in different ethnic groups (Malays, Indians and Chinese) there (28,29,30).

This study also displayed that smoking status of the respondents exhibited associated CAD risk. A status of cigarette smoking habit was also documented in this study. Data demonstrated that 33% of CAD respondents were in fact, smokers. Smoking tobacco is often described as being linked to early atherosclerosis onset. As such, smoking upregulates the inflammatory genes that can accelerate the atherosclerotic process (31). On the contrary, a majority of non-CAD respondents comprised over 50% population of smokers. This increased risk factor of developing CAD may have been avoided by giving significant attention to lifestyle changes. Multiple reports and studies showed that despite being free from CVD history, responders who smoke had higher long-term risk of fatality rate related to various CVD disorders than those who did not smoke (32).

Overweight status, reflected in increased BMI value and abdominal obesity was found to be a significant CAD risk, which was consistent with previous studies (33,34,35). Obesity has often been associated as a risk factor for CVD, along with hypertension, dyslipidemia and diabetes. Obesity has even been classified as an independent risk factor for CVD/CAD (36). Besides, the SBP status for both groups of CAD and non-CAD was prehypertensive (120 – 139 mmHg), with significant differences between both groups. This can be explained by the fact that CAD respondents had a higher SBP status, which can be referred to as hypertension incidence. Individual within these blood pressure range can as well be justified as high risk of atherosclerotic progression (37).

Lipid profile of CAD respondents tends to show low HDL with elevated TC, LDL and TGs levels. Studies have shown a strong and consistent association between chronic systemic inflammation and susceptibility to CVD (26,27). HDL acts as a cholesterol transport function for cholesterol and was significantly lower in respondents with CAD compared to non-CAD. HDL is an important risk factor for CVD because it works as a cholesterol transporter from the blood vessel in the form of chylomicrons to the liver. Lower HDL can lead to the association risk of the plaques in the blood vessel together with high TG and LDL. Furthermore, TGs has shown a positive relationship with cause of death in CVD patients which was associated with poor BMI and nutritional status (38).

In this study, the tHcy levels were found to be 26.8% higher in CAD respondents than in non-CAD. It has been suggested that the alteration of tHcy metabolism in obese is susceptible due to the dysregulation of the MTHFR and cSHMT enzymes (39,40). Hcy and cysteine play a vital role in the synthesis of essential fatty acids for regeneration of nervous systems (40). The imbalance of cysteine or Hcy levels were due to lack of vitamin B12 in patients dietary source (41,42). Nevertheless, further statistical analysis on the interaction of MTHFR and cSHMT mutation enzymes with Hcy levels, required in this study to validate whether high Hcy levels can associate with CAD/CVD.

These findings led to a speculation that an association exists between high Hcy with CAD risk. There are possible explanations for the relationship between Hcy and CAD. Hcy is a sulfur-containing amino acid that is a precursor for methionine and cystathione. Elevated Hcy levels are not only considered a risk factor for CAD, but also linked to chronic kidney disease (43), depression (44) and neurodegenerative disorder (45). In-vivo with in-vitro perspective study showed that high Hcy levels had toxic effects on the vasculature, with manifestations of medial remodelling, adventitial inflammation and endothelial injury which in turn is promoting the reactive oxidative species production (46).

CONCLUSION

The case-control study viewpoints clarified the indicators of CAD associated factors development, including age progression, ethnicity, BMI, systolic blood pressure, TC, TG, HDL, LDL, smoking status, exercise activity, abdominal obesity, family history of CVD, and tHcy. The levels of tHcy were significantly elevated in patients with CAD in comparison to those without CAD. It suggested that the variability of tHcy levels across different countries and its positive association to CAD renders it as a relevant prospective biomarker in assessing the risk of CAD and CVD.

ACKNOWLEDGEMENT

The authors would like to express their profound gratitude to all respondents who participated in this research. The deepest appreciation to the National Heart Institute (NHI) Kuala Lumpur, Malaysia, Universiti Putra Malaysia (UPM), and the National Blood Bank, Malaysia (NBB).

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