ORIGINAL ARTICLE

Plasma Haptoglobin as A Potential Biomarker for Coronary Artery Disease in Young Hypertensive Adults

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ABSTRACT

INTRODUCTION: Uncontrolled hypertension is one of the recognized risk factors for coronary artery disease (CAD) in young adults, commonly underestimated owing to the young age. A novel biomarker to improve CAD risk assessment and hypertension management should be identified for this cohort. Thus, we had conducted a study to investigate plasma concentration and the role of haptoglobin in young hypertensive adults in the establishment of premature acute myocardial infarction (AMI). **MATERIALS AND METHODS:** A total of 120 male adults aged between 18 to 45 years enrolled into this cross-sectional study, divided into control, hypertensive, and acute myocardial infarction (AMI) groups. Blood samples were collected from all subjects, plasma concentrations of haptoglobin measured using enzyme-linked immunosorbent assays, and other CAD risk factors including high sensitivity C-reactive protein (hs-CRP) levels were analyzed. **RESULTS:** Plasma concentration of haptoglobin in the AMI group was the highest compared to hypertensive and control group (290.63±99.90 vs. 208.47±112.93 vs. 170.02±108.11 ng/ml, p<0.006). There was a significant association between AMI and plasma haptoglobin concentration in hypertensive subjects independent of other known CAD risk factors (OR: 0.985, 95% CI 0.973-0.997, p=0.017). There was positive correlation between plasma haptoglobin and hs-CRP (r=0.0370, p<0.001). **CONCLUSION:** Plasma haptoglobin is a potential biomarker to identify young hypertensive adults who are at risk of developing CAD.

Keywords
Haptoglobin, Biomarker, Coronary Artery Disease, Hypertension, Young Adults

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INTRODUCTION

Acute myocardial infarction is the commonest clinical presentation of coronary artery disease (CAD) primarily affecting older individuals. However, there has been a recent increase in the incidence of acute myocardial infarction (AMI) in young adults, indicating that being young is no longer a protective factor against CAD.[1-2] A cut-off age of 45 years is commonly used to define young AMI in many studies.[3-8] Young AMI patients predominantly were male and hence, the diagnosis of AMI at this productive age has significant socioeconomic consequences, including the loss of valuable human capital, an increased financial burden on the nation, and a strain on limited public health care resources.[5-8] The Fourth Universal Definition of myocardial infarction defines AMI as myocardial ischemia followed by myocardial necrosis with an increase and/or decrease in plasma troponin level, accompanied with prolonged chest pain, or/and abnormal electrocardiography (ECG) changes.[9] The diagnosis of ST-elevation myocardial infarction (STEMI) is based on the presence of myocardial necrosis identified by elevation of ST segment in ECG tracing while non ST elevation myocardial infarction (NSTEMI) is when there is no ST-elevation in ECG tracing, yet there is an elevation of troponin. The third category is unstable angina which refers to myocardial ischemia without myocardial necrosis as the troponin value is below the decision limit for AMI. Aside from smoking, hypertension is recognized as a main risk factor for AMI development in young adults.[10-15] Unfortunately, young adults experiencing their first AMI are more likely to have

untreated hypertension than older AMI patients.[16-17] Early detection and effective treatment of hypertension are crucial in reducing the risk of future CAD in this cohort. However, accurate hypertension management in young adults is more challenging than older cohort due to inaccurate cardiovascular disease (CVD) risk assessment tools specific to this age group. For example, in the Framingham CVD risk assessment, a young adult with hypertension is more likely to be underestimated in terms of their 10-year risk of developing CVD owing to young age is generally considered a protective factor.[18-19] As a result, young adults with hypertension are more likely to be classified in the low CVD risk category with less aggressive intervention and therapy.

Therefore, discovery of novel biomarker (s) to improve risk stratification of CAD in young hypertensive adults is essential for more accurate hypertension management in this cohort. Haptoglobin is an acute phase reactant protein in response, synthesized mainly in the liver and it is the subject of research as a potential biomarker of many diseases including artherosclerosis.[20] In human, haptoglobin genes is polymorphic in which there are three structural alleles that control the formation of three major types of phenotypes; homozygous Hp1-1, Hp2-2 and heterozygous Hp2-1.[20] A prior study has shown that the plasma concentration of haptoglobin was significantly elevated in young adults with AMI compared to controls in response to the inflammation reaction in the pathophysiology of AMI.[21]

Besides, a prospective study spanning eleven years showed that plasma haptoglobin was as effective as total cholesterol in predicting AMI, stroke, and heart failure.[22] However this study focused on elderly population with a limited scope of comparing AMI patients with controls.[22] Currently, the role of plasma haptoglobin as CAD biomarkers in young adults with hypertension has not been explored. Therefore, this study aimed to compare the plasma haptoglobin concentration in young AMI patients, hypertensive subjects, and control subjects to unlock the potential of haptoglobin as a biomarker of CAD in young cohort.

MATERIALS AND METHODS

Study Design and Subject Recruitment

We conducted a cross-sectional study among male young adults aged between 18 to 45 years involving a total of 120 subjects; 40 subjects in control, hypertensive and AMI group respectively. Control and newly diagnosed hypertensive subjects were recruited during health screening at selected outpatient clinics and higher education institutions. During the first meeting, we measured the first blood pressure and invited the potential subjects to the second meeting for repeat blood pressure examination and fasting blood taking procedure. For each meeting, blood pressure was measured twice on the non-dominant arm in a seated position with arm and back supported, using a calibrated automated blood pressure machine (Omron HEM-7139) after a 5-minute rest with a 10-minute interval and the average was computed.

The normotension was defined as systolic blood pressure (SBP)<120 mmHg and diastolic blood pressure (DBP)<80 mmHg, whereas hypertension was defined as SBP≥140 mmHg or DBP≥90 mmHg for these two consecutive meetings.[18] Hypertensive subjects were defined as newly diagnosed patients prior taking any antihypertensive drugs while control subjects were defined as healthy young male adults with normal blood pressure and no history of chronic illness such as diabetes, neoplasms, infections, and autoimmune diseases. This study excluded subjects who were taking long term medications including antihypertensive drugs to minimise the co-founding factors.

Meanwhile for AMI subjects recruitment, we recruited young AMI patients who presented at the Emergency Department of Hospital Tengku Ampuan Afzan (HTAA), Kuantan, Malaysia with newly-diagnosed ST elevated or non-ST elevated myocardial infarction and have yet to receive thrombolysis or percutaneous coronary intervention. The diagnosis criteria of AMI included the presence of prolonged chest pain, typical changes in a 12-lead electrocardiogram (ECG), and/or elevated serum creatine kinase-MB and the diagnosis was verified by the

on-site Emergency Physician. The troponin was not used as part of the diagnosis criteria of AMI in this study as this biomarker level was not a standard protocol at the clinical site of recruitment and it was not available for all patients. The study assessed the socio-demographic data and CAD risk factor profiles in all subjects. Body mass index (BMI) was derived from the body weight divided by the height squared (kg/m^2). Prior to recruitment and any intervention, informed consents were obtained from all subjects. This study was conducted in accordance with the Declaration of Helsinki, and approved by the Ministry of Health's Medical Research and Ethics Committee (MREC), ID NMRR-16-2572-32869.

Blood Sample Collection

We collected 10-15 ml of blood samples from subjects in control and hypertensive groups, and at acute presentation of AMI subjects at the emergency department respectively. The blood was centrifuged at 2500 revolutions per minute for ten minutes. The plasma was collected into microcentrifuge tubes. A proportion of blood were stored in a -80 ^{0}C freezer until further analysis while another proportion of blood were sent to an accredited lab for measurement of total cholesterol, fasting blood glucose and high sensitivity C-reactive proteins (hs-CRP). These biochemistry parameters for AMI patients were recorded based on the inpatient blood investigation results.

Enzyme-Linked Immunosorbent (ELISA) Analysis

To quantify the plasma haptoglobin concentrations, we used enzyme-linked immunosorbent assays (ELISA) kit (Elabscience®, USA) following the manufacturer's protocol. First, 100 µl of sample was pipetted into antibody pre-coated microplate wells and incubated at 37 ^{0}C for 90 min. The protein-antibody complex was then detected by a biotinylated detection antibody that was specific to the targeted protein and successively recognized by Avidin-Horseradish Peroxidase (HRP) conjugate. Next, 90 µl of substrate reagent was pipetted into each well to bind to the protein-biotinylated detection antibody-HRP conjugate. This enzyme-substrate reaction ceased with the addition of 50 µl stop solution and the optical density (OD) was measured at 450 nm wavelength using a microplate reader (Infinite 200 PRO, TECAN, Switzerland). We interpolated the protein concentration of each sample from the standard curve that was run in the same plate.

Statistical Analysis

Categorical data was presented as n (%). We evaluated the normality of all numerical data using histogram, skewness and kurtosis test. Normally distributed data was presented as means ± standard deviation while non-normal distributed data was presented as median (interquartile range (IQR)). We compared the mean haptoglobin concentration between the three groups using One Way ANOVA with Tukey post hoc test and Kruskal-Wallis H test as appropriate. The association between young AMI and plasma haptoglobin in young hypertensive patients was evaluated using multivariate logistic regression that were adjusted for other known CAD risk factors. The correlation between plasma haptoglobin and hs-CRP was determined using Spearman's test as. All statistical analysis were performed using IBM® SPSS® Statistics 24 software. We considered $p<0.05$ as significant.

RESULTS

Socio-Demographic and CVD Risk Factor Profiles

Table 1 demonstrates socio-demographic and CAD risk factor profiles of all subjects in this study. AMI patients had the highest mean age, percentage of current smokers, numbers of smoking pack years and fasting blood glucose compared to hypertensive and control subjects. Meanwhile, the hypertensive subjects had the highest mean of waist circumference, BMI and total cholesterol compared to AMI patients and control subjects. The level for hs-CRP was not normally distributed and the median was the highest in AMI group, followed by hypertension and control group; 16.00 (IQR 26.75) vs. 2.70 IQR (4.45) vs. 1.10 (IQR 2.70) ng/ml, $p< 0.001$.

Plasma Concentrations of Haptoglobin

We found a significant higher plasma concentration of haptoglobin in AMI subjects compared to control subjects; 290.63 ± 99.90 vs. 170.02 ± 108.11, $p<0.001$ and hypertensive subjects; 290.63 ± 99.90 vs. 208.47 ± 112.97, $p=0.006$ as demonstrated in Figure 1.

Table I: Baseline characteristics of all subjects in the study

Variables	Control		Hypertension		AMI		p-value
	(n = 40)		(n = 40)		(n = 40)		
	Mean	±SD	Mean	±SD	Mean	±SD	
Age, years	33.1	±5.3	35.1	±6.8	[a]37.4	±5.0	0.002
Waist circumference, cm	87.0	±8.4	[a]98.3	±13.1	95.8	±16.9	0.018
BMI, kg/m^2	24.80	±3.50	[a]30.13	±4.89	[a]29.62	±6.88	<0.001
Total cholesterol, mmol/L	5.95	±1.02	6.09	±1.22	5.64	±1.56	0.506
SBP, mmHg	113	±6	[a]148	±10	[a]133	±9	<0.001
DBP, mmHg	73	±5	[a]99	±8	[a]87	±14	<0.001
Hs-CRP ng/ml*	1.10	(2.70)	2.70	(4.45)	[b]16.00	(26.75)	<0.001
Smoking, pack-year*	4.2	(5.2)	5.0	(14.3)	[b]16.0	(13.9)	0.015
Fasting glucose, mmol/L*	4.85	(0.48)	5.05	(0.60)	[b]6.30	(1.11)	<0.001
BMI status	n	(%)	n	(%)	n	(%)	
Underweight/Normal (<23 kg/m^2)	11	(27.5)	0	(0)	7	(17.5)	
Overweight (23-27.5 kg/m^2)	21	(52.5)	13	(32.5)	11	(27.5)	
Obese (> 27.5 kg/m^2)	8	(20.0)	27	(67.5)	22	(55)	
Smoking status							
Current	10	(25.0)	16	(40.0)	33	(82.5)	
Ex-smoker	7	(17.5)	4	(10.0)	4	(10.0)	
Non smoker	23	(57.5)	20	(50.0)	3	(7.5)	

Data expressed as mean (standard deviation, SD), *median (interquartile range, IQR) or numbers (%).
[a] $p < 0.05$ when compared to the control using One Way ANOVA and Tukey post hoc test.
[b] $p < 0.05$ when compared to the control using Independent Samples Kruskal-Wallis H test.
BMI: body mass index, BMI classification follows Asia-Pacific BMI Classification, SBP: systolic blood pressure, DBP: diastolic blood pressure,

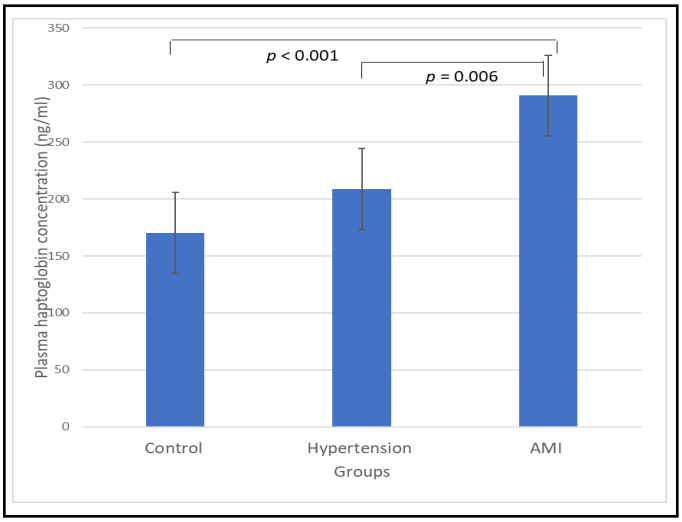

Figure 1: Plasma concentration of haptoglobin in control, hypertensive and AMI group. Data is expressed as mean ± standard deviation and analyzed using One Way ANOVA and Tukey post hoc test. p <0.05 was considered as significantly different. AMI: Acute myocardial infarction.

To determine the risk factors of AMI among the hypertensive young adults, we analyzed the data using multivariate logistic regression analysis among the hypertensive and AMI subjects with the latter as the reference category as shown in Table II.

We found that plasma haptoglobin is an independent risk factor of AMI in hypertensive young adults (OR: 0.985, 95% CI 0.973-0.997, p=0.017). One ng/ml decrease of plasma haptoglobin hypertensive young adults reduces the risk of AMI by 1.5%. Apart from plasma haptoglobin, smoking (p=0.001, OR=13.9) and fasting blood glucose (p=0.002, OR=0.338) were other significant covariates of AMI in young hypertensive adults. Additionally, plasma concentration of haptoglobin was moderately correlated with the concentration of hs-CRP using Spearman correlation test (r=0.370, p<0.001).

Table II: Association between AMI and plasma concentration of haptoglobin in hypertensive young adults

Variables	B	SE	P	OR	95% CI	
					Lower	Upper
Age	-0.009	0.051	0.855	0.991	0.896	1.096
Smoking	2.634	0.826	0.001*	13.932	2.763	70.259
Total cholesterol	0.494	0.253	0.050	1.639	0.999	2.689
Fasting blood sugar	-1.084	0.354	0.002*	0.338	0.169	0.677
Body mass index	0.089	0.067	0.184	1.093	0.959	1.247
Plasma haptoglobin	-0.015	0.006	0.017*	0.985	0.973	0.997

Logistic regression analysis with AMI as the reference category. B = estimated coefficient, SE: standard error, P: p value, OR: odd ratio, CI: confidence interval. *Statistically significant at p < 0.05, 95% confidence interval.

DISCUSSION

Hypertension is an important risk factor for CAD. Due to the increasing incidence of CAD among young adults, it is important to identify a potential biomarker that can be used to predict CAD in this cohort. In our previous proteomic discovery phase study, we found that plasma

haptoglobin expression was upregulated in AMI compared to controls in a young adult population.[23] In this study, we added another group of subjects which was the hypertensive young adults and we found that plasma haptoglobin in AMI to be significantly higher in AMI compared to the hypertensive and control group respectively. Overall, our findings were in agreement with another study among general adult population, comparing 359 subjects with CAD and 83 control. [24] Apart from the different age group, our inclusion criteria for the CAD subjects also differed. While we studied subjects with newly-diagnosed AMI in acute setting, Lee and co-workers recruited stable CAD patients who presented for elective angiography.

Taking into account all the findings, it could be proposed that plasma haptoglobin has started to be highly expressed early on in the CAD development, especially in the chronic low-grade inflammation underlying the hypertension, leading to AMI occurrence, and continue to remain elevated in the post-acute setting. At the moment, the exact duration of how long the plasma haptoglobin remains elevated after a CAD event warrants further investigation. In order to assess the association between plasma haptoglobin and AMI among young hypertensive subjects, we analysed the hypertensive and AMI subjects in a separate analysis. After adjusting for other CAD risk factors, we found that plasma haptoglobin in hypertensive young adults was significantly associated with AMI. As such, we proposed that plasma haptoglobin to have a potential role as a CAD biomarker in young hypertensive subjects.

Our findings prove to add to the body of knowledge as previous studies have named adiponectin and endocan as potential CAD biomarkers in hypertensive patients.[25-26] Nevertheless, our findings are specifically tailored to young adults aged 18-45 years which have yet to be explored. While adiponectin and endocan are principally implicated in endothelial dysfunction pathogenesis and vascular remodeling, haptoglobin is an acute phase protein highly expressed in inflammation.[27] Haptoglobin synthesis is induced by interleukin-6, which is a key pro-inflammatory cytokine.[27-29] In support of this, we examined the association between plasma haptoglobin with an established biomarker of vascular inflammation. We found positive correlation between plasma haptoglobin and hs-CRP in the current study, replicating the finding of another study by Yao and Yang[3] among young adults. Altogether, we could deduce that haptoglobin contributes to vascular inflammation namely hypertension and atherosclerosis, in the pathophysiology of CAD. CAD has wide range of clinical presentation from asymptomatic, to AMI presentation which carries high mortality and morbidity. Because of the high burden of CAD, it is in the best interest to detect it early to allow timely preventive measures and intervention. However, due to its complexity in pathophysiology, we could argue that relying on a single biomarker for early disease prediction is inadequate.[31]

We proposed that plasma haptoglobin could be utilized together with hs-CRP as a panel of biomarkers to contribute to a risk score or logarithm in assessing early development of CAD. The essential role of inflammation in young patients with CAD is further supported by the significantly high number of active smokers among AMI patients in the current study. It is deduced that tobacco smoking causes a 20-25% increase in leukocytes and inflammatory markers such as interleukin 6 and hs-CRP. [32] This study has also demonstrated that young hypertensive subjects were associated with higher BMI and waist circumference compared to control and AMI subjects. These findings proposed that hypertension in young adults is secondary to metabolic syndrome.[33] The clustering of CAD risk factors among young hypertensive adults highlights the importance of early blood pressure control, adequate CAD risk assessment and early intervention.

The presence of metabolic syndrome among young hypertensive patients would further put them at higher risk of early CAD. More so, there are increasing evidences relating haptoglobin and metabolic syndrome.[34-39] This study has several limitations. Our study design was cross-sectional. Hence, we could not delineate a cause-and-effect mechanism in the present study. Furthermore, since the AMI subjects were from a single centre, our findings may not be applicable to the general population. We proposed a multi-centered prospective cohort study with a larger sample size to better represent the general young adult population, and detect the smaller difference between

groups which we may miss due to smaller sample size. Also, our study did not stratify the AMI group into those with pre-existing hypertension and non-hypertensive AMI group. Having this additional information would add on more information on the role of haptoglobin in the pathophysiology of AMI in hypertensive young adults. Further study would include the identification of the subjects' specific phenotypes of haptoglobin as it is proposed that phenotype of haptoglobin could be utilized to evaluate the individual predisposition of a person to various diseases.

CONCLUSION

Plasma concentration of haptoglobin in young hypertensive adults was significantly associated with AMI independent of other known CVD risk factors. Therefore, it serves as a potential biomarker to discriminate young hypertensive adults with high risk of developing premature CAD. The positive correlation of haptoglobin with hs-CRP enhanced the potential of haptoglobin as an inflammatory marker in the establishment of CAD in young adults.

CONFLICT OF INTEREST

There are no conflicts of interest.

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