ORIGINAL ARTICLE

Urinary Apolipoprotein A1 and its Potential as a Biomarker for Coronary Artery Disease in Young Adults

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ABSTRACT

INTRODUCTION: Very few studies have focused on exploring the utilisation of urinary protein biomarkers to improve the risk stratification of coronary artery disease (CAD) in young adults. Apolipoprotein A1 (ApoA1) as a primary constituent protein of High-density Lipoprotein (HDL) known to modulate cholesterol metabolism exhibits promising properties to be used as a protein biomarker, specifically for CAD in young adults. Thus, this study is aimed to evaluate the potential of urinary ApoA1 as a urinary biomarker of CAD in young patients with acute myocardial infarction (AMI). **MATERIALS AND METHOD:** This case-control study recruited 40 newly diagnosed AMI patients and 40 healthy control subjects aged 18–45. Urine samples were collected from all subjects. Once centrifuged, the supernatant was collected and stored at -80 °C until further analysis. The urinary concentration of ApoA1 was quantified using the ApoA1 Enzyme-linked Immunosorbent Assay (ELISA) kit according to the manufacturer's protocol. All subjects' risk factors were determined and documented, such as smoking status, Body Mass Index (BMI), blood pressure, plasma total cholesterol, and glucose levels. **RESULTS:** The mean age of AMI patients was higher than the controls; 37.1 1 ± 5.2 and 31.6 ± 8.1 years respectively. The mean urinary concentration of ApoA1 of AMI patients was significantly higher than the controls (12. 442 ± 3.571 vs. 10.067 ± 5.606 ng/mL ($p<0.05$). Following an adjustment to other conventional CAD risk factors, there was an insignificant association between urinary excretion of ApoA1 and AMI in young adults (Odd Ratio (OR)=3.123, 95% CI: 0.756–1.015, $p>0.05$). **CONCLUSION**: A significant elevation of urinary excretion of ApoA1 in AMI young adults demonstrated its potential use as a urinary protein biomarker for CAD in young adults.

Keywords
Apolipoprotein A1, urinary, biomarkers, coronary artery disease, young adults.

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INTRODUCTION

Acute myocardial infarction (AMI) is the most frequently reported clinical manifestation of coronary artery disease (CAD) and continues to burden the healthcare system in developing countries, including Malaysia. In fact, it is concerning to notice the rising trend of AMI among the younger population.[1-2] According to the most recent National Cardiovascular Disease–Acute Coronary Syndrome (NCVD-ACS) Registry report, a quarter of AMI patients aged 50 years and younger were admitted to tertiary hospitals across Malaysia.[2] The majority of patients were male and are the breadwinner in the family, which contributes to more significant socio-economic and psychological impacts on individuals, families, and communities.[2-3] The use of conventional assessment tools, such as the Framingham Risk Score of CAD for risk stratification, is less relevant since young AMI patients commonly exhibit minimal traditional cardiovascular disease risk factors.[4-5] Thus, identifying new protein biomarker(s) to detect early development of atherosclerosis, specifically in this age group is vital to improving risk stratification, diagnosis, and prognostication of CAD.

Urine is not merely a waste product of the urinary system. In fact, it is an easily accessible and valuable biological sample, similar to plasma, for protein biomarker research

and clinical investigations. Modifications in urine protein profiles may reflect the pathological process of diseases, including CAD.[6] For instance, Brown et al.[7] reported 190 individual proteins differently expressed in urine samples of CAD cases compared to the controls. Htun et al.[8] also revealed 75 ACS-related urinary peptides, but none of these panel proteins was verified individually in the verification phase. Meanwhile, the association between cardiovascular diseases and several urinary proteins, namely amino-terminal propeptide of type-III procollagen (PIIINP), neutrophil gelatinase-associated lipocalin (NGAL), and α1-microglobulin (A1M), were previously evaluated in older patients.[9] However, urinary biomarkers may not be useful for older adults as they often have co-morbidities and polypharmacy, which could affect the excretion of urinary proteins. Conversely, urinary biomarkers are preferable in younger cohorts with minimal confounding factors.[10]

One of the new protein biomarkers studied for CAD in young adults is Apolipoprotein A1 (ApoA1), a key component of High-density Lipoprotein (HDL).[11] A previous study demonstrated an increase in the plasma concentration level of ApoA1 among young patients with AMI, which is associated with an inflammatory response secondary to cardiac muscle injury.[11] Meanwhile, other studies have reported the atheroprotective property of ApoA1, given its essential role in the reverse cholesterol transport process.[12-13] However, many of these studies utilised plasma samples. Thus, the role of urinary ApoA1 in CAD development in young adults remains unexplored. Furthermore, a systemic review meta-analysis reported an increased level of ApoA1, which is associated with an early diagnosis of bladder cancer and its prognosis.[14] It is essential to establish the potential of ApoA1 as a urinary marker for the diagnosis and prognosis of CAD in young adults, as ApoA1 is the primary functional component of HDL. In addition, ApoA1 is a urinary low molecular weight protein of about 28 kDa that is typically filtered into the urine. The discovery of CAD urinary biomarker (s) would potentially serve as a non-invasive screening approach to recognise early significant coronary atherosclerosis among young adults who are usually considered healthy and rarely require medical care. Therefore, this study investigated the concentration of ApoA1 in urine samples of young patients with AMI compared to control patients and evaluated the association between urinary ApoA1 and CAD in young adults.

MATERIALS AND METHODS

Study Design and Subject Recruitment

This case-control study involved AMI patients aged 18–45 years who were admitted to the Emergency Department of Hospital Tengku Ampuan Afzan (HTAA) Kuantan, Malaysia and diagnosed with ST-elevated Myocardial Infarction (STEMI) or non-ST-elevated Myocardial Infarction (NSTEMI). The diagnosis criteria include prolonged chest pain associated with at least one of these criteria; distinctive changes in 12-lead Electrocardiogram (ECG) or/and serum creatine kinase-MB elevation. Subjects with chronic illnesses, such as diabetes mellitus, neoplasm, infections and autoimmune diseases, and/or on long-term medications were excluded from the study to minimise the confounding factors. Meanwhile, control subjects consist of healthy volunteers recruited during health screenings at outpatient clinics. Prior to the commencement of the study, informed consent was obtained from each subject. Subsequently, socio-demographic data were documented, followed by the assessment of the cardiovascular risk factors profiles, such as Body Mass Index (BMI), waist circumference, smoking status, blood pressure, plasma total cholesterol, and fasting sugar levels. This research was conducted according to the Declaration of Helsinki, and the study protocol was approved by the Ministry of Health Medical Research and Ethics Committee (MREC) (ID NMRR-16-2572-32869).

Urine Sample Collection

Following the AMI diagnosis, approximately 30 mL of mid-stream urine was collected from patients in the Emergency Department before the administration of thrombolysis or Percutaneous Coronary Intervention (PCI) procedure. Urine samples from the control subjects were also collected during a health screening appointment at an outpatient clinic. The collected urine samples were centrifuged at 2500 revolutions per minute (rpm) for 10 minutes. The supernatant was collected and stored at -80 °

C for further analysis.

Enzyme-linked Immunosorbent Assays (ELISA) Analysis

The urinary concentration of ApoA1 was quantified using the Human ApoA1 Enzyme-linked Immunosorbent Assays ELISA kit (Abcam, United States). Briefly, the standard working solution and urine sample (50 µL) of AMI patients and controls were pipetted into the pre-coated antibody ELISA microplate wells, followed by two hours of incubation. Then, the plate was washed five times with 200 µL of 1x Wash Buffer manually. Next, 50 µL of 1x Biotinylated Apolipoprotein A1 Antibody was added to each well and incubated for one hour to detect the ApoA1-antibody complex. After that, the microplate was washed again, as mentioned above. Next, 50 µL of 1x SP Conjugate was added to each well, and the mixture was incubated for 30 minutes. Once the microplate was washed, 50 µL of Chromogen Substrate was pipetted into each well and incubated in ambient light for 25 minutes until the optimal blue colour density developed. The enzyme-substrate reaction was halted by the addition of 50 µL Stop Solution. The optical density of the reaction was measured using a microplate reader at a wavelength of 450 nm (Azure Biosystems, China). A standard graph was constructed, with the x-axis representing the standard concentration and the y-axis representing the optical density values. Finally, the exact concentration of ApoA1 in each sample was calculated using the equation of the constructed graph.

Statistical Analysis

The baseline characteristics of all participants' CAD risk factor profiles and the urinary concentration of ApoA1 were presented as mean and Standard Deviations (SD). In order to compare quantitative data between case and control groups, the independent Student's t-test was used for numerical data, while the Chi-Squared test was used for categorical data. In addition, the association between urinary ApoA1 and AMI in young patients was evaluated using multivariable logistic regression, which was adjusted for other known CAD risk factors, including age, BMI, systolic and diastolic blood pressure, total cholesterol, and fasting plasma glucose levels.

RESULTS

Socio-demographic and Risk Factor Profiles

Table 1 presents the baseline characteristic of CAD risk factors profiles of all subjects in this study. In general, males were dominant in both groups, with significantly higher mean age and fasting glucose levels in the AMI group than in control (37.1 ± 5.2 vs 31.6 ± 8.1, p=0.012) and (6.5±1.5 vs 5.4±1.9 mmol/L, p=0.020), respectively. The percentage of active smokers was significantly higher in the AMI group, with 85% compared to only 30% in the control group (p<0.001). Conversely, there was no significant difference in BMI, waist circumference, blood pressure, and total plasma cholesterol levels.

ELISA Analysis of Urinary Apolipoprotein A1

The urinary concentration of ApoA1 was normally distributed. Figure 1 shows that the urinary concentration of ApoA1 in the AMI group was significantly higher than in the control (12.442 ± 3.571 vs 10.067±5.606 ng/mL, p=0.025). The urinary concentration of ApoA1 was also higher in smokers than non-smokers (11.439±5.017 vs 10.753±4.650, p=0.533).

Table 1: Baseline Characteristic of all Subjects

Variables	AMI n = 40		CONTROL n = 40		*P*-value
Male, %	90		70		0.001
Current smoker, %	85		30		0.001
Age, years	37.1	(5.2)	31.6	(8.1)	0.012
Waist circumference, cm	91	(15)	92	(14)	0.660
Body mass index, kg/m²	29	(6)	28	(6)	0.690
SBP, mmHg	135	(22)	130	(21)	0.352
DBP, mmHg	88	(18)	87	(14)	0.733
Fasting plasma glucose, mmol/L	6.5	(1.5)	5.4	(1.9)	0.020
Total Cholesterol, mmol/L	5.9	(1.7)	5.7	(1.1)	0.573

Data were analysed by using the Chi-squared test for categorical data and Independent Student's t-Test for numerical data and values are expressed as mean (standard deviation). AMI = acute myocardial infarction, SBP=systolic blood pressure, DBP=diastolic blood pressure.

However, when adjustments were made for other established risk factors of CAD, such as age, BMI, blood pressure, cholesterol and fasting glucose levels, the logistic regression in Table 2 shows that ApoA1 was insignificantly associated with AMI in young adults [OR = 3.123, 95% CI: 0.756–1.015, p=0.077).

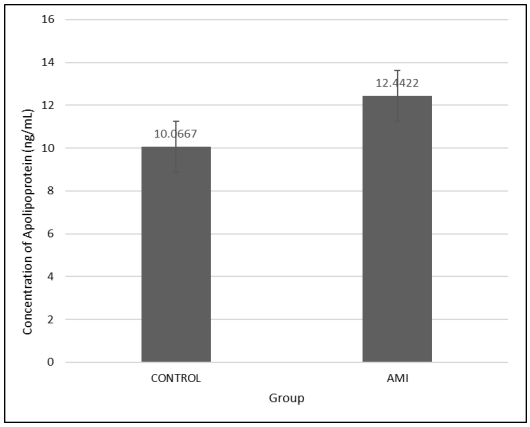

Figure 1: Mean urinary concentrations of Apolipoprotein A1 in 40 control subjects and 40 AMI patients. Data was analysed using an Independent Student's t-Test, $p = 0.025$. AMI = acute myocardial infarction.

DISCUSSION

ApoA1 is a principal constituent of HDL and is known to be inversely proportional to the risk of developing CAD.15 The cardioprotective effect of HDL has been primarily attributed to the presence of ApoA1 and its role in reverse cholesterol transport.[15] However, the findings from this study revealed an increased excretion of ApoA1 in urine samples of young AMI patients compared to the control, which agrees with the rising level of plasma ApoA1 in young AMI patients compared to the control from previous studies.[11,16] The elevated excretion of ApoA1 protein in urine samples of young AMI patients was associated with the dysfunctional HDL in the establishment of CAD. As proposed by Ertek[17], ApoA1 constitutes about 70% of HDL components (mainly in the proteome) and is affected by inflammation, consequently modifying the HDL function.

Table 2: Association between Apolipoprotein A1 and AMI in young adults

Variable	B	AOR	95% CI		*P* value
			Lower	Upper	
Age	0.138	4.615	0.768	0.988	0.032
Body mass index	0.072	0.120	0.849	1.361	0.547
Total cholesterol	0.442	0.778	0.790	3.062	0.201
Systolic BP	0.041	1.917	0.680	1.017	0.166
Diastolic BP	0.067	2.554	0.985	1.161	0.110
Fasting plasma glucose	0.002	0.001	0.680	1.465	0.991
Urinary ApoA1	0.133	3.123	0.756	1.015	0.077

Multivariate logistic regression analysis; AMI=acute myocardial infarction, B=coefficient, BP= blood pressure, AOR=adjusted odd ratio, CI= confidence interval

Hence, this study's findings highlighted the significance of monitoring HDL functions rather than only focusing on its level.

The cardioprotective properties of ApoA1 may change under specific circumstances. For instance, Srivastava et al.[18] concluded that oxidative stress and inflammation promoted HDL dysfunction, triggering a greater risk of cardiovascular disease. The result is consistent with that of Clark et al.[19], who found that an increased ApoA1 in urine samples of children with kidney diseases was associated with the inflammatory response. Besides, Dardeer et al.[14] proposed ApoA1 as a highly specific and sensitive urinary biomarker for bladder cancer. Thus, it was postulated that the overexcretion of ApoA1 in young AMI patients in this study resulted from dysfunctional HDL secondary to a significant inflammatory process in the establishment of CAD in young patients.

Comparatively, young AMI patients differ from elderly patients in terms of risk factor profiles, clinical presentations, and the main pathological process involved.[20-21] Inflammation plays a significant role in young AMI adults mainly because they are strongly associated with cigarette smoking compared to elderly patients, who are more implicated with hypercholesterolemia, hypertension, and diabetes mellitus.[21-22] This is consistent with the finding in this study, as observed with the insignificant difference between the AMI group and control subjects regarding BMI, total cholesterol levels, and systolic and diastolic blood pressure. Additionally, this study identified that young AMI patients who smoke exhibit the main CAD risk factors. Past studies have shown that smoking promotes atherogenesis through chronic inflammation response.[23] Apart from that, smoking stimulates and activates systemic and local immune systems by increasing the white blood cell and proinflammatory cytokines, accelerating the formation of atherosclerotic plaques.[23-24] The enhanced inflammation and oxidative stress condition would alter the properties of ApoA1 from cardioprotective to atherogenic.[25] Consistently, this study has shown that the urinary concentration of ApoA1 was higher in smokers than non-smokers. Since ApoA1 is vulnerable to oxidative modifications by cigarette

smoking, ApoA1 becomes dysfunctional and loses its atheroprotective properties in smokers.[26]

Notably, the findings of this study are not without limitations. As such, the relatively small sample size may contribute to the insignificant association between ApoA1 and AMI in young adults in the multivariate analysis. Therefore, a prospective study utilising a bigger sample size may provide more accurate information to evaluate the role of urinary ApoA1 in establishing CAD compared to a case-control study design. The present study also lacked HDL data. Hence, this parameter should be included in future studies to evaluate its correlation with ApoA1.

CONCLUSION

This study highlighted the potential utilisation of urinary ApoA1 as a biomarker for CAD in young AMI adults. The elevated urinary excretion of ApoA1 in young AMI patients suggests its practical use as an alternative non-invasive approach to predict the early onset of significant coronary atherosclerosis among young adults. This finding is considered a valuable pilot study to evaluate the role of urinary ApoA1 biomarkers in cardiovascular disease.

CONFLICT OF INTEREST

There is no conflict of interest.

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