International Journal of PharmTech Research
CODEN (USA): IJPRIF, ISSN: 0974-4304, ISSN(Online): 2455-9563
Vol.9, No.11, pp 01-09, 2016

Association Between Caffeine with Cyp450 1a2 And Il-6 Levels in Acehnese Who Suffer from Coronary Heart Disease

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Abstract : Coffee, a main source of caffeine, has controversial effects on cardiovascular health. We evaluated the association of caffeine levels with the levels of Cytochrome P450 Subfamily 1A2 (CYP1A2) and interleukine-6 (IL-6) in Acehnese coffee drinker with coronary heart disease (CHD) compared to who do not consumed coffee (non-coffee drinkers). Fourty eight Acehnese male with CHD consisted of 24 coffee drinkers and 24 non-coffee drinkers participated in this case-control study which was conducted in Cardiology Division of Internal Medicine and Heart Catheterization Laboratorium of dr. Zainoel Abidin General Hospital, Banda Aceh. Afternoon blood samples were collected to determine the levels of caffeine, CYP1A2 and IL-6. Data obtained were analyzed for the differences and its correlations. Plasma levels of caffeine and IL-6 in coffee drinkers were significantly higher compared to that in non-coffee drinkers. Although CYP1A2 levels in coffee drinkers appeared to be higher compared to that non-coffee drinkers, but it was not statistically significant (p=0,06). There was a significant correlation between the levels of caffeine with the levels of IL-6 (r=0.36; p=0.01), but there was no significant correlation between the levels of caffeine with the levels of CYP1A2 (r=0.23; p=0.12). The present study demonstrated that the levels of caffeine and IL-6 differ based on coffee consumer habit, not for CYP1A2 levels. It is only a significant association between the levels of caffeine with the levels of IL-6.
Keywords : plasma caffeine level; CYP1A2 level; IL-6 level; coronary heart disease.

Introduction

Coronary heart disease (CHD) is the leading cause of death in the world, with a mortality rate of one in five men and one in six women. The average mortality rate from ischemic heart disease in the world has increased [1,2]. More than three-quarters of cardiovascular disease deaths take place in developing countries. Of the 16 million deaths under the age of 70 years old due to non-communicable diseases, 82% in countries of low and middle income and 37% are caused by cardiovascular disease [3]. In Indonesia, according to the Basic Health

Research (Riset Kesehatan Dasar, Riskesdas) in 2007 showed CHD was ranked as the third leading cause of death after stroke and hypertension [4], in 2013 CHD is still in the top 10 most non-communicable diseases [5]. Based on data from the highest referral Zainoel Abidin Regional General Hospital Banda Aceh, Indonesia, the number of patients visit in cardiac clinic and patients undergoing cardiac catheterization increases every year. The latest data for 2015 heart clinic visits ranged 100-150 subjects per day, while those undergoing cardiac catheterization procedures scheduled 70-80 patients per month [6]. A large amount of cohort studies addressed coffee consumption and risk of CHD yielded inconsistent results. A meta-analysis study showed that habitual moderate coffee consumption showed significantly lower CHD [7]. Although an assumption that an increased risk for cardiovascular disease in heavy coffee drinkers has been rejected it becomes interesting because of the cultural habit of Acehnese is to drink coffee even as rice companion [8].

Coffee is a major source of caffeine (1,3,7-trimethylxanthine), a purine alkaloid from plants. Caffeine is a competitive antagonist of neurotransmitter adenosine on adenosine receptors, it has also an important role in pharmacology and it is used as a supplementary substance in medicaments [9]. Caffeine is mainly metabolized by the polymorphic cytochrome P450 1A2 (CYP1A2) enzyme [10,11].

It has been demonstrated that several different pro-inflammatory cytokines including interleukin-6 (Il-6) are associated with CHD risk independent of conventional risk factors and in an approximately log-linear manner [12,13,14]. Because inflammation may play a role in the pathogenesis of CHD, therefore the present study aims to determine the association of the levels of plasma caffeine with CYP1A2 and Il-6 levels in Acehnese coffee drinkers who suffer from CHD.

2. Material and Methods

2.1 Subjects

Forty eight adult Acehnese (24 coffee drinker and 24 non-coffee drinkers) partisipated in this case control study. They were adult male patients (i.e. outpatient and inpatient) suffering from CHD who visit the Zainoel Abidin General Hospital in Banda Aceh. Subjects must meet the admission criteria, through history, physical examination, electrocardiography (ECG), echocardiography (optional), treadmill test (optional) and underwent cardiac catheterization. Subjects who met the study criteria were given a medical information sheet and an explanation of the purpose of research before signing a written informed consent.

The present study was conducted from February 2015 untill the collection of the samples completed in the Division of Cardiology, Internal Medicine Department, Faculty of Medicine, University of Syiah Kuala, Banda Aceh and Cardiac Catheterization Laboratory at dr. Zainoel Abidin Hospital of Banda Aceh, after obtaining approval from the Health Research Ethical Committee of Medical School, University of North Sumatra and a permission from the director of the Zainoel Abidin General Hospital, Banda Aceh.

The acceptance criteria are as follows; Acehnese adult male subjects at least two generations of lineage with age $\geq$ 30 years. Drink at least 2 cups of coffee a day for cofee drinkers and do not drink coffee as a comparison. Criteria for rejection are as follows; patients who have undergone arterial bypass surgery, impaired renal function (creatinine> 2 mg / dl, CrCl <60ml / min), Diabetes Mellitus Type 2 or were taking an oral antidiabetic / insulin, hypertension or were taking anti-hypertensive drugs, dyslipidemia, overweight / obesity, consuming medicines (paracetamol, clozapine, haloperidol, olanzapine, propranolol, tacrine, theophylline, and zolmitriptan).

2.2 Laboratory examination

After coronary angiography examination, blood samples (20 cc) were drawn in the afternoon (5 pm) for analysis in a laboratory examination. Caffeine levels were measured by using the Capillary Gas Chromatograph GC-2010 Plus made in Shimadzu Corporation, 2014, Japan. Mean within-day coefficients of variation for caffeine levels were < 6%.

Plasma IL-6 protein (IL-6) levels were assayed using the Quantikine® HS Human IL-6 Immunoassay Kit (R&D Systems, Minneapolis, MN 55413, USA; Cat: HS600B, Lot: 329440, ED: 02.11.2015) as per

package protocol. Detection range of the IL-6 assay was 0,016 to 11,0 pg/mL with the intra- and inter-assay coefficients of variation were < 7.8% and < 9.6% respectively.

The examination of CYP1A2 level is using ELISA KIT Human Cytochrome P450 1A2 Cusabio products Biotech Co., Ltd., P.R. China Cat: CSB-EL00639HU, Lot: J04119354, ED: 20 September 2015 (CYP1A2 R / Cusabio). The range of the calibration standard used is 15.6 - 1000 pg / mL, detection limit: 3.9 pg / mL, with the intra- and inter-assay coefficients of variation were < 8% and < 10% respectively.

2.3. Data Analyis

The results are expressed as means ± SDs for normally distributed continuous variables, as medians and ranges for non-normally distributed continuous variables. Obtained data were then analyzed for the difference of means using unpaired T-test if the data of the two groups in normal distribution, and Mann Whitney test for data not normal distribution. The correlation of caffeine, CYP1A2 and Interleukin-6 plasma levels were determined by using Pearson correlation test if both data is normally distributed, if not normally distributed by using Spearman correlation test. A P value <0.05 was considered significant level.

3. Result

Basic characteristics of CHD patients who were coffee drinkers and non-coffee drinkers are shown in table 1.

Both groups cofee drinkers and non-coffee drinkers have similar chracteristics in all the basic test data except post prandial blood sugar levels, however the post-prandial blood sugar levels in both groups were within normal limits (<200 mg/dl) (Table 1).

Table 1. Basic characteristics of coronary heart disease patients coffee drinkers and non-coffee drinkers.

	Coffee Drinkers			**Non-coffee Drinkers**			**P**
	Mean	**SD**	**Data Normality Test***	**Mean**	**SD**	**Data Normality Test***	
Age	56,65	10,54	0,19	55,77	8,56	0,71	0.741
IMT	23,27	4,52	0,01	24,67	3,06	0,69	0.360
Systolic BP	115,00	15,03	0,01	116,54	9,36	0,01	0.292
Diastolic BP	73,85	7,52	0,00	76,15	6,97	0,00	0.207
Hemoglobin	13,02	1,94	0,00	14,15	1,63	0,01	0.749
Eritrocyte	5,02	0,64	0,30	5,12	0,58	0,22	0.564
Leukocyte	9,66	2,70	0,23	8,58	2,23	0,50	0.121
Trombocyte	290,58	88,54	0,00	253,77	68,52	0,04	0.351
Hematocrite	42,21	3,70	0,80	42,90	4,88	0,14	0.569
ESR	26,77	23,46	0,00	18,42	7,89	0,98	0.557
Fasting blood sugar levels	103,38	11,94	0,08	98,77	16,35	0,36	0.251
2 hours post prandial blood sugar levels	156,12	27,94	0,20	133,15	30,37	0,01	0.002
Cholestrol	177,15	27,50	0,79	180,00	45,34	0,15	0.785
LDL	100,92	24,48	0,10	86,31	35,19	0,17	0.088
HDL	40,50	9,94	0,32	47,85	28,93	0,00	0.927
Age	56,65	10,54	0,19	55,77	8,56	0,71	0.741
IMT	23,27	4,52	0,01	24,67	3,06	0,69	0.360
Triglycerides	159,08	56,70	0,02	148,88	55,73	0,59	0.481
Ureum	29,27	12,16	0,00	30,17	13,36	0,29	0.503
Creatinin	1,13	0,41	0,00	1,21	0,83	0,00	0.742
Ulric acid	7,23	1,62	0,01	7,01	1,22	0,00	0.783

Table 2. Differences of the levels of caffeine, CYP1A2 and IL-6 in coffee drinkers and non-coffee drinkers who suffer from CHD.

Variables	Coffee drinkers			Non-coffee drinkers			P
	Mean	SD	Median	Mean	SD	Median	
Caffeine (µg/ml)	2,94	4,16	1,27	0,21	0,59	0,00	0,00**
CYP1A2 (pg/mL)	950,52	174,60	938,30	851,85	180,29	840,90	0,06*
IL – 6 (pg/mL)	2,57	1,18	2,46	1,90	1,23	1,55	0,01**

Unpaired T test *; Mann Whitney **

Table 2 shows significant differences in the levels of caffeine (2,94 µg/ml vs 0,21 µg/ml) and IL-6 (2,57 pg/mL vs 1,90 pg/mL), but not for CYP1A2 levels (950,52+174,6 pg/mL vs 851,85+180,29 pg/mL) between coffee drinkers and non-coffee drinkers. Plasma caffeine levels in coffee drinkers gained a median level of 1.27 while non-coffee drinkers have median levels of 0.00.

The relationship of plasma caffeine levels with the number of cups of coffee in coffee drinkers suffered from CHD is shown in Figure 1. Figure 1 shows that 19 subjects drink 2 cups per day, 4 subjects drink 3 – 4 cups per day and one subject drinks ≥ 5 cups per day with the highest caffeine levels (i.e. 18.65 µg/ml). One of subject drinks 2 cups coffee per day had the most high caffeine levels (i.e. 4.95 µg/ml) and 3 subjects had not detectable caffeine levels. There is a significant relationship (r=0.71; P<0,001) between the number of coffee drinks with the caffeine level collected at afternoon time.

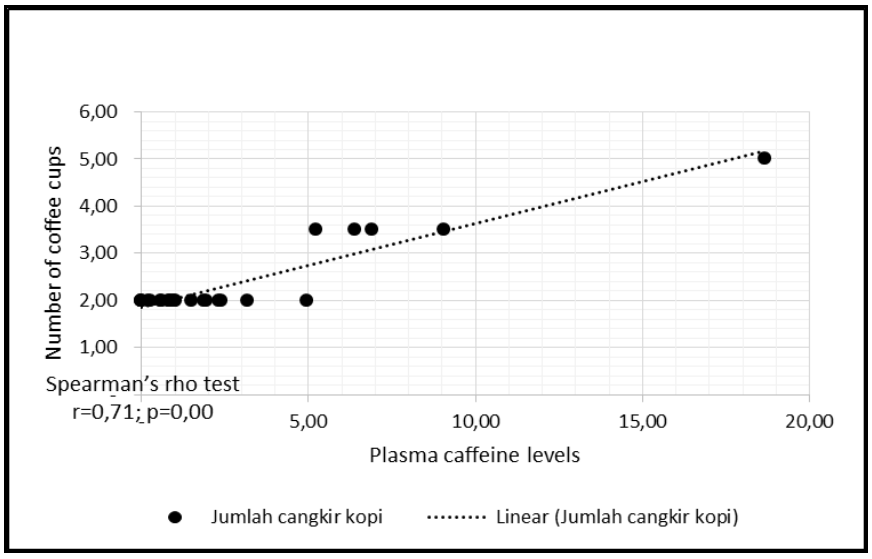

Figure 1. The relationship of plasma caffeine levels with the number of cups of coffee in coffee drinkers suffered from CHD.

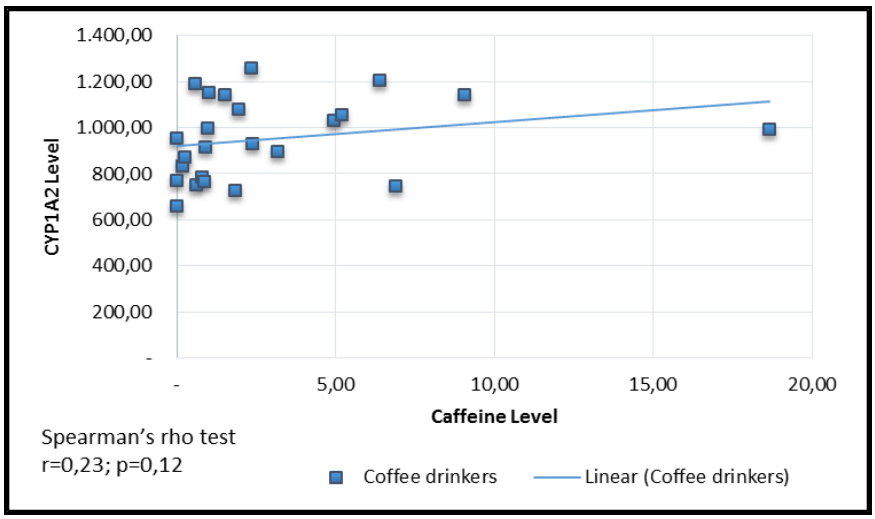

Figure 2. The relationship of plasma caffeine levels with the levels of CYP1A2 in coffee drinkers who suffer from CHD.

Figure 2 illustrates the relationship between the levels of plasma caffeine and the levels of CYP1A2 in coffee drinkers who suffer from CHD, however it is not statistically significant (r=0,23; p=0,12).

Figure 3 illustrates the significant relationship (p=0,01) of plasma caffeine levels with IL-6 levels in coffee drinkers who suffer from CHD, but the relationship is not strong enough (r= 0,36).

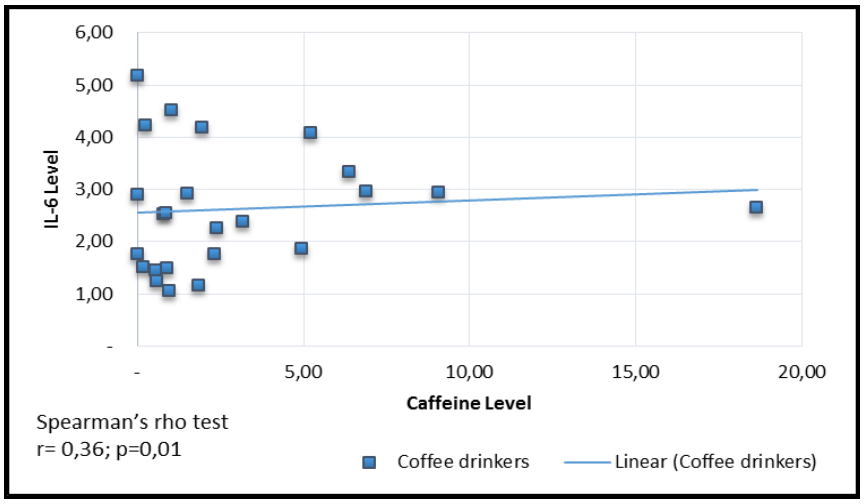

Figure 3. The relationship of plasma caffeine levels with the levels of IL-6 in coffee drinkers who suffer from CHD.

There is no significant relationship between the levels of CYP1A with IL-6 in both coffee and non-coffee drinkers. However, there is different pattern of relationship between the levels of CYP1A2 and IL-6, i.e. positive relationship in coffee drinkers (r=0,21; p=0,31) and inverse relationship in non-coffee drinkers (r= -0,32; p=0,11).

4. Discussion

Various methods have been described in determination of caffeine concentration in various biological and pharmaceutical preparations [15], ie. Spectrophotometry, High Performance Liquid Chromatography (HPLC), Reverse Phase High Performance Liquid Chromatography (RP-HPLC), and FTIR. In the present study the concentration of caffeine in blood plasma was determined by Gas Chromatography/Mass Spectrophotometry (GC/MS).

Blood sampling of coffee drinkers for determining the caffeine concentration was collected in the afternoon. the caffeine content of the beverage or intake of caffeine-containing foods correlates proportionately to the increase plasma concentration of caffeine in which the correlation is better in the afternoon (17:00) or evening (21:00) than in the morning plasma samples [16]. The present study demonstrated a good correlation (r=0.71; P<0,001) between the number of cups of coffee consumed with the caffeine concentration collected at afternoon time in coffee drinkers who suffer from CHD (Figure 1).

Up to now, the number of cups of caffeine-containing beverages consumed has often been used as an index of caffeine intake [17,18]. Compared with the light-to-absent coffee consumption (<1 cup/day in the United States or ≤2 cups/day in Europe), moderate coffee consumption (>1 or 2 cups daily, respectively) was associated with significantly lower rates of CHD in the entire group of men and women with an RR of 0.87 (p = 0.001) [19]. Most of our coffee drinkers were moderate consumption. It is worth to note that caffeine absorption and exposure from coffee and energy drinks is similar irrespective of beverage temperature or rate of consumption [20].

Thorn et al. (2012) report that caffeine is almost completely metabolized in human body, only 3% or less is excreted in unchanged form in the urine [21]. The main pathway of metabolism in humans (70-80%) through 3-N-demethylation into paraxanthine also known as 1,7-dimethylxanthine [16,21]. Cytochrome P450 (CYP)1A2 is responsible for ~95% of caffeine metabolism in humans. A study in Danish mono and dizygotic twins showed that the non-polymorphic 3-N-demethylation of caffeine catalyzed by CYP1A2 is subject to approximately 70% genetic control [11]. The Coffee and Caffeine Genetics Consortium findings among European and African-American adults reinforce the role of caffeine in mediating habitual coffee consumption and may point to molecular mechanisms underlying inter-individual variability in pharmacological and health effects of coffee [22]. Chow et al found a great variability of CYP1A2 activity measured by caffeine/paraxanthine ratio ranging from 1.31 to 21.4 [23]. Table 2, showed levels of CYP1A2 coffee drinkers and non-coffee drinkers who suffer from CHD appear to be different, CYP1A2 levels were higher in coffee drinkers compared to non-coffee drinkers, but it is no statistically significant. A previous report demonstrated that increased plasma caffeine levels is followed by elevated levels of CYP1A2 [21] as also demonstrated in the present study. In premenopausal women CYP1A2 activity was also positively related to caffeine intake (P = 0.007) [24]. In the present study it also showed that CYP1A2 levels increase with plasma caffeine levels in subjects suffered from CHD, but this association was not statistically significant (r=0,39; p=0,06) (Figure 2).

Results from animal studies have shown that caffeine is an inducer of CYP1A2 [25]. This suggests that CYP1A2 induction by caffeine may be in part responsible for caffeine tolerance. However, caffeine did not induce CYP1A2 expression in primary human hepatocytes at a concentration attained by ordinary coffee drinking. Because of higher CYP1A2 levels in coffee drinkers (Table 2) and positive relationship of caffeine with CYP1A2 levels (Figure 2), is there an autoinduction in caffeine metabolism by CYP1A2 in human being? Factors other than CYP1A2 induction by caffeine likely contribute to development of caffeine tolerance in humans [25]. Actually, near complete tolerance, in terms of both humoral and hemodynamic variables, developed over the first 1-4 day of caffeine consumption [26]. Various agents may induce CYP1A2 activity. An animal study demonstrated that insulin can induce CYP1A2 activity [27]. Smoking is able to induce CYP1A2 activity, and as expected smokers had higher CYP1A2 activity than did non-smokers. The increased CYP1A2 activity in the smoking population was probably due to induction of the CYP1A2 gene via the Ah receptor causing an increase in the concentration of CYP1A2 protein [28]. The limit of our study is not to take into account the smoking habit of all participant.

It was previously demonstrated a diurnal variation in CYP1A2 enzyme activity. Perera et al observed diurnal variation of CYP1A2 activity in South Asians, resulting in lower enzyme activity in the evening. A higher CYP1A2 activity (mean ± standard deviation) was found in the morning (0.52 ± 0.17) when compared with evening (0.47 ± 0.17) ($n = 23,\ P < 0.05$) [29]. All data of CYP1A2 levels in the present study were determined from blood samples collected at afternoon time. Therefore, there is assumed no diurnal effect on data obtained in the present study.

Zampelas et al found that the levels of IL-6 in men who consumed coffee > 200 ml/day are approximately 50% higher compared to men who were not drinking coffee [30]. In the present study IL-6 levels in coffee drinkers were significantly higher compared to that in non-coffee drinkers (2,57 pg/mL vs 1,90 pg/mL) (Table 2). A previous study of Wedick et al demonstrated that consumption of caffeinated coffee by regular

coffee consumers (≥ 2 cups/day) increased IL-6 concentrations (1.95 pg/mL) compared with consuming no coffee (1.22 pg/mL) [17]. The increase of IL-6 in coffee drinkers of our study (35%) is much less compared to the report of Wedick et al (60%), but the level of IL-6 in coffee drinkers of our study (2,57 pg/mL) appears much higher compared to the report of Wedick et al (1.95 pg/mL). The present study found a significant association (p=0,01) of plasma caffeine levels with IL-6 levels. There was an increase IL-6 levels with the increase of caffeine levels in coffee drinkers who suffer from CHD, but the assosiation is not strong enough (r= 0,36) (Figure 3).

Plasma concentrations of IL-6 reflecting the intensity of plaque inflammation and the risk of plaque rupture [14]. Several studies has reported that the increased levels of plasma IL-6 in patients with unstable angina or stable angina compared with healthy individuals might be used as prognostic value in patients with cardiovascular disease. An increased IL-6 in plasma is a strong and consistent value as a risk factor for cardiovascular events. Cesari et al and Ammirati et al found that levels of cytokine IL-6 increased in STEMI determine clinical outcome worse than STEMI levels of cytokines IL-6 is low [12,13]. An animal study demonstrated that coffee intake did not affect the production of IL-6 and TNF-alpha induced by LPS, then it was concluded that moderate coffee intake is not a risk factor for atherogenesis [31].

Frye et al found a striking inverse relationship between IL-6 plasma concentrations with the metabolism of caffeine (CYP1A2**)** in patients with congestive heart failure [32]. It means the levels of IL-6 wil increase when the levels of CYP1A2 decrease. This phenomena was only demonstrated in non-coffee drinkers, there is increasing of the IL-6 levels with decreasing of the CYP1A2 levels, but statistically not significant (r= - 0,32; p=0,11), while in coffee drinkers there is a positive relationship between the levels of IL-6 with the levels of CYP1A2. This evidence might also support the assumption of the present of an autoinduction of caffeine metabolism in coffee drinkers.

In conclusion, the present study demonstrated that plasma levels of caffeine and IL-6 in coffee drinkers were significantly higher compared to that in non-coffee drinkers. Although CYP1A2 levels in coffee drinkers appeared to be higher compared to that non-coffee drinkers, but it was not statistically significant (p=0,06). The present study demonstrated that the levels of caffeine and IL-6 differ based on coffee consumer habit, not for CYP1A2 levels. There was a significant correlation between the levels of caffeine with the levels of IL-6 (r=0.36; p=0.01), but there was no significant correlation between the levels of caffeine with the levels of CYP1A2 (r=0.23; p=0.12).

Limitation

Limitations to the study include that age, diet, and smoking habits factors are not considered in this study that are likely to affect the results.

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